☐ Intended for Regulatory Submission
☐ Export Controlled

Document Cover Page
Dow AgroSciences Confidential

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	10001126-5051-4	080058	04-MAY-10

Document Title

A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

Author(s) and ID(s)

Rotondaro, S. L. and Balcer, J. L.

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-2,4-D DMA salt

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)

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)
					4-May-10
					GLP Status
					GLP

Analytical Methods Only:

Method Type	Analyte(s)

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]-2,4-D Applied to AAD-1 Corn, 2008

DATA REQUIREMENTS

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

AUTHORS

S. L. Rotondaro, J. L. Balcer

STUDY COMPLETED ON

04 May 2010

PERFORMING LABORATORIES

Regulatory Laboratories—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

Research For Hire
1696 South Leggett Street
Porterville, California 93257

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

LABORATORY STUDY ID

080058

A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

SUMMARY

[^{14}C]-2,4-D (2,4-dichlorophenoxy acetic acid dimethylammonium salt) was applied to plots of AAD-1 maize at the maximum seasonal rate of 3.36 kg a.e./ha (three applications, each 1.12 kg a.e./ha at pre-emergence, V4, and V8). The maize was grown outdoors to maturity at Research For Hire. Plot maintenance simulated typical cultural practices.

Forage was collected on 7 August 2008 (70, 42, and 30 days after the first, second, and third applications, respectively). Forage contained 3.531 µg acid equivalents/g. Mature grain, cobs, and fodder were collected 3 September 2008 (97, 69, and 57 days after the first, second, and third applications, respectively). At maturity, the grain contained 0.040 µg a.e./g. The mature cobs and fodder contained 0.026 µg a.e./g and 5.563 µg a.e./g, respectively. A portion of each sample was sequentially extracted with neutral solvent then methanolic base and the non-extractable residue was subjected to bound residue determinations such as pectin, acid-detergent fiber, lignin, and cellulose isolation. Starch was isolated from a separate portion of grain.

In the mature grain, a portion of the radioactive residue was identified as parent (7% of the TRR, 0.003 mg a.e./kg). The major radioactive component in forage and fodder was parent, 67.5% and 51.3% of the TRR (2.383 mg a.e./kg and 2.855 mg a.e./kg), respectively. Glucose-conjugated phenol was identified in forage and fodder. Approximately 30% of the TRR in grain (0.012 mg a.e./kg) was associated with starch. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin.

STUDY TITLE

A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

DATA REQUIREMENTS

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

AUTHORS

S. L. Rotondaro 317-337-3508
[SRotondaro@dow.com]
J. L. Balcer

STUDY COMPLETED ON

04 May 2010

PERFORMING LABORATORIES

Regulatory Laboratories—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

Research For Hire
1696 South Leggett Street
Porterville, California 93257

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

LABORATORY STUDY ID

080058

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: 2,4-D

Title: A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

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: March 18, 2010

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

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
ENV/MC/CHEM(98)17, Paris January 26, 1998

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, and reference standard X11963417 was not GLP analyzed prior to use.

D. Fonseca
Sponsor
Dow AgroSciences LLC

March 18, 2010
Date

D. Fonseca
Submitter
Dow AgroSciences LLC

March 18, 2010
Date

S. L. Rotondaro
Study Director/Author
Dow AgroSciences LLC

04 May 2010
Study Completion Date

Dow AgroSciences Quality Assurance Unit
Good Laboratory Practice Statement Page

Study ID: 080058

Title: A Nature of the Residue Study with [14C]-2,4D Applied to AAD-1 Corn, 2008

Study Initiation Date: 25-March-2008 **Study Completion Date:** 04-May-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-Mar-2008	Protocol Review
24-June-2008	29-September-2008	Procedures for the Determination of Ammonium Acetate Extractable Ca, Mg Na & K in Soil (NUT.02.12) (Agvise Laboratories)
24-June-2008	29-September-2008	Organic Matter- Walkley Black Method (NUT.02.09.06) (Agvise Laboratories)
11-August-2008	29-September-2008	Raw Data audit (Agvise Laboratories)
10-October-2008	13-October-2008	Extraction
07, 12-16, 19-23 – April-2010	23-April-2010	Raw Data & Report Review and Test Substance Container Verification

AUDITS CONDUCTED BY RESEARCH FOR HIRE QA ARE LISTED ON THE QUALITY ASSURANCE STATEMENT OF THE SUBREPORT IN APPENDIX A AND AUDITS CONDUCTED BY ABC LABORATORIES, INC. QA ARE LISTED ON THE QUALITY ASSURANCE STATEMENT OF THE SUBREPORT IN APPENDIX B.

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.

Val Gartner

Val Gartner, RQAP-GLP
Dow AgroSciences, Quality Assurance

04-May-2010
Date

SIGNATURE PAGE

S. L. Rotondaro
Author
Dow AgroSciences LLC

04 May 2010
Date

J. L. Baker
Co-author/ Mass spectrometry report
Dow AgroSciences LLC

18 Mar 2010
Date

M. Ma
Reviewer
Dow AgroSciences LLC

18 Mar 2010
Date

T. C. Blewett
Reviewer, Science Leader
Dow AgroSciences LLC

19 Mar 2010
Date

M. J. Hastings
Reviewer, Technical Leader
Dow AgroSciences LLC

18 MAR 2010
Date

A. S. McGibbon
Global Leader, Fate and Metabolism and
Analytical Sciences
Dow AgroSciences LLC

19 Mar 2010
Date

STUDY PERSONNEL

Title:	A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008
Principle Investigator:	B. Turner (Research For Hire)
Principle Investigator:	C. Chickering (ABC Labs)
Study Director:	S. L. Rotondaro
Analysts:	S. L. Rotondaro, K. P. Smith, A. Tiefert (Kelly Scientific), J. Gesell (Kelly Scientific)

TABLE OF CONTENTS

TABLE OF CONTENTS (CONT.)

Page

TABLE OF CONTENTS (CONT.)

TABLE OF CONTENTS (CONT.)

A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008

1.0 SUMMARY ABSTRACT

[^{14}C]-2,4-D (2,4-dichlorophenoxy acetic acid dimethylammonium salt) was applied to AAD-1 maize at the maximum seasonal rate of 3.36 kg a.e./ha (three applications, each 1.12 kg a.e./ha at pre-emergence, V4, and V8). The maize was grown outdoors to maturity at Research For Hire. Plot maintenance simulated typical cultural practices.

Forage was collected on 7 August 2008 (70, 42, and 30 days after the first, second, and third applications, respectively). Forage contained 3.531 µg acid equivalents/g. Mature grain, cobs, and fodder were collected 3 September 2008 (97, 69, and 57 days after the first, second, and third applications, respectively). At maturity, the grain contained 0.040 µg a.e./g. The mature cobs and fodder contained 0.026 µg a.e./g and 5.563 µg a.e./g, respectively. A portion of each sample was sequentially extracted with neutral solvent then methanolic base and the non-extractable residue was subjected to bound residue determinations such as pectin, acid-detergent fiber, lignin, and cellulose isolation. Starch was isolated from a separate portion of grain.

In the mature grain, a portion of the radioactive residue was identified as parent (7% of the TRR, 0.003 mg a.e./kg). The major radioactive component in forage and fodder was parent, 67.5% and 51.3% of the TRR (2.383 mg a.e./kg and 2.855 mg a.e./kg), respectively. Glucose-conjugated phenol was identified in forage and fodder. Approximately 30% of the TRR in grain (0.012 mg a.e./kg) was associated with starch. Extensive metabolism of 2,4-D was observed as demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as starch, pectin, and lignin. Residue levels are summarized below.

Sample	2,4-D		DCP		DCP-conjugate	
Forage	% TRR	ae mg/kg	% TRR	ae mg/kg	% TRR	ae mg/kg
Neutral Extraction	62.6%	2.209	1.0%	0.035	16.1%	0.567
Methanolic Base Extraction	4.9%	0.174	0.4%	0.013	0.9%	0.033
Total Identified	67.5%	2.383	1.4%	0.048	17.0%	0.600

	2,4-D		DCP		DCP-conjugate	
Grain	% TRR	ae mg/kg	% TRR	ae mg/kg	% TRR	ae mg/kg
Neutral Extraction	7.1%	0.003	ND	ND	ND	ND
Methanolic Base Extraction	--	--	--	--	--	--
Total Identified	7.1%	0.003	ND	ND	ND	ND

	2,4-D		DCP		DCP-conjugate	
Fodder	% TRR	ae mg/kg	% TRR	ae mg/kg	% TRR	ae mg/kg
Neutral Extraction	43.2%	2.403	1.0%	0.057	21.6%	1.199
Methanolic Base Extraction	8.1%	0.452	0.1%	0.006	2.6%	0.144
Total Identified	51.3%	2.855	1.1%	0.063	24.2%	1.344

-- Not analyzed
ND = not detected

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 corn (event 474) following the maximum seasonal application of ^{14}C-2,4-D. One plot was treated with three applications, including one pre-emergence and two foliar spray applications. The ^{14}C-2,4-D was formulated as a soluble liquid (SL), the current proposed commercial formulation, and applied at a target rate of 3.36 kg a.e./ha (three applications, each 1.12 kg a.e./ha at pre-emergence, V4, and V8).

2.1.2. Relevant History and Background Information

2,4-D (2,4-dichlorophenoxy acetic acid dimethylammonium salt), is an herbicide developed primarily for control of broad-leaved weeds (dicotyledons) in cereal crops (monocotyledons) and a variety of other crops and on non-crop land. 2,4-D is a selective, systemic herbicide. It is a synthetic auxin.

When applied as the salt or as an ester formulation, 2,4-D undergoes rapid hydrolysis to the acid. Previous studies (*1*) have demonstrated that 2,4-D is readily degraded by soil microorganisms. The side chain is cleaved to the phenol which is further oxidized to a catechol derivative. The labeled carbon is postulated to degrade completely to $^{14}CO_2$ and enter the metabolic pool via the Krebs cycle.

Hydroxylation, decarboxylation, cleavage of the acid side chain, ring opening, and conjugation with amino acids or sugars are the major metabolic pathways of 2,4-D in plants (*1*).

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).

2.1.4. Guideline Deviations

None

2.2. Justification of Study Application Rate

The maximum seasonal application rate for 2,4-D is 3363 g a.e./ha (4048 g a.i./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

The radiolabeled test substance of the technical product was obtained from the Dow AgroSciences Specialty Synthesis Group. See Figure 1 for structure, radiolabel position, and nomenclature. Physico-chemical properties of 2,4-D can be found in Table 1.

3.1.2. Reference Substances

Non-radiolabeled 2,4-D and available metabolite standards were acquired from the Test Substance Coordinator at Dow AgroSciences for use as reference standards for chromatographic and mass spectral comparison. Non-radiolabeled test material was also used to dilute the specific activity of the dosing solution. All reference standards were received in solid form. Structures, purity, ID numbers, chemical names and abbreviations for reference substances are presented in Figure 2. Reference standards were prepared in acetonitrile or acetonitrile/water at

a concentration of approximately 1 mg/mL. Bulk 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

A soluble liquid 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. 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-2,4-D DMA, nominally 5.8 mCi, was received from Specialty Synthesis. The entire sample was diluted to 10-mL in methanol, using a volumetric flask. Aliquots (0.025 mL) were diluted in 20-mL volumetric flasks, using water to dilute to volume. Aliquots of the dilutions were analyzed by LSC and to determine the actual amount of radioactive test substance received as well as the purity (Figure 4).

The specific activity of the ^{14}C-2,4-D DMA, was adjusted by combining non-radiolabeled 2,4-D DMA with the majority of the ^{14}C-2,4-D DMA test substance solution. Individual test substance samples were prepared for each of the three applications by dispensing the appropriate amount of this diluted specific activity solution. The individual test substance samples were evaporated 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 0.190 mL of formulation blank F1222-208. 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 appropriate vial of application solution was allowed to come to ambient temperature, transferred to a spray container, the original sample vial rinsed multiple times with water, and the final spray solution brought to a known volume with water. Aliquots were taken to determine the concentration 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. 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.

Mature crop was harvested on 3 September 2008. 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 prior to 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 a hammer and/or a Robot Coupe with dry ice. The milling of the 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. Data results from the combustion assay of the treated samples can be found in Appendix B.

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 10 g of homogenized forage and fodder, or 20 g grain or cobs, were extracted with approx. 50 mL of 90/10 methanol/water (100 mL for grain and cobs), 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 extract using less extraction solvent. The volume of the extract was measured and triplicate aliquots (0.25-2.0 mL) 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 (forage) or concentrated after increasing the pH ≥9 (grain, cobs), or C_{18} SPE procedure described in Section 3.9.4 (grain, fodder), then analyzed by HPLC as described in 3.9.5.

A second set of replicates of grain (approximately 20 g) were extracted in a similar manner, first using hexane (75 mL, Polytron homogenization 2 minutes during first extraction only) followed by 90/10 methanol/water (3 x 75 mL). The extracts were pooled by solvent per sample, the

volume of each measured and triplicate aliquots (3.0 mL) were analyzed by liquid scintillation counting as described in Section 3.9.2. Additional aliquots of the methanol/water extracts were cleaned-up using the Strata-X SPE procedure described in Section 3.9.3, then analyzed by HPLC as described in 3.9.5.

3.8.2. Methanolic Base Extraction (EX2)

The tissue remaining after the neutral extraction (3.8.1) was further extracted with approx. 25 or 50 mL of 90/10 methanol/1.0 N NaOH, as described in Figure 5 and Table 4. The mixture was heated to approximately 50 ºC while shaking for one hour (not shaken for forage), 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. Grain (replicates C & D only) was further extracted with approximately 50 mL methanol/water (90/10), shaking 30 minutes without heat, pooling the three extracts. The volume of the combined extract was measured and triplicate aliquots (0.5-3.0 mL) were analyzed by liquid scintillation counting as described in 3.9.2. Additional aliquots of the forage and fodder extracts were cleaned-up using the C_{18} SPE procedure described in 3.9.4, then analyzed by HPLC as described in 3.9.5.

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

The tissue remaining after the second sequential extraction (Section 3.8.2) was weighed. Triplicate aliquots (0.1 g) were analyzed by oxidative combustion 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 (*2*).

3.8.4.1. Pectin Solubilization

The pectin substances in the NRR were solubilized using ethylenediaminetetraacetic acid (EDTA), 50 mM in 50 mM pH 4.5 buffer (*3*) as described in Figure 6 and the second page of

Table 4. An aliquot (1 g) of the non-extractable tissue (Section 3.8.3) was sonicated or Polytron homogenized at 10K rpm for approximately two minutes, then heated (approx. 70 ºC) with 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 determined and triplicate aliquots (3.0 mL) 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 (*4*). First, the solids were transferred to flasks and covered with water (40 mL). Sodium chlorite (1.25 g $NaClO_2$) and glacial acetic acid (150 µL) were added to each solid sample, stirred, and heated in a hot water bath (approx. 65 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. 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 (*5*) and is also described in Figure 6 and the second page of Table 4. The pellet from the lignin extraction step (Section 3.8.4.2) was then 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 an 80-100 ºC oven, 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* (6). One replicate of each of the following was used for the isolation procedures: fresh, unextracted grain (replicates E & F); 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 dried in a 60-70 ºC oven overnight 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 when not in the process of analysis.

3.8.6. Metabolite Identification Procedures

Metabolites were isolated from the methanol/water extracts of forage tissue. First, aliquots (75 mL) of the extract replicates were purified via C_{18} SPE, using the procedures described in Section 3.9.4, eluting with 15 mL methanol/water/1.0 N NaOH (80/20/1, v/v/v). Because 12-18% of the radioactivity was found in the load/wash, the SPE procedure was repeated with this phase (without concentration). The combined eluate was concentrated to dryness on the TurboVap (40 ºC and 10 psi nitrogen) and reconstituted in methanol (5.0 mL). Aliquots of each phase were analyzed by LSC.

The remainder of the methanol eluate was dried onto silica (approx. 1 g) and added to the top of a silica SPE (1 g). The silica was eluted with a series of non-polar to polar solvents (25 mL each), including: 1) hexane; 2) hexane/chlorobutane/diethylamine (DEA), 90/10/0.5; 3) hexane/chlorobutane/DEA, 50/50/0.5; 4) chlorobutane/DEA, 100/0.5; 5) chlorobutane/ethyl acetate/DEA, 90/10/0.5; 6) chlorobutane/ethyl acetate/DEA, 50/50/0.5; 7) ethyl acetate/DEA 100/0.5; 8) ethyl acetate/methanol/water/DEA, 80/20/2.0/0.5; 9) methanol/water/DEA, 95/5/0.5; 10) methanol/acetic acid, 95/5. Fractions 3, 8, 9, and 10 were individually concentrated to dryness (TurboVap, 40 ºC) and reconstituted in water/acetonitrile (80/20, v/v, 1.0 mL).

Concentrated fractions 8 and 9 above for both replicates were combined and again purified by a single silica SPE. The procedure was similar as above, except a narrower range of elution solvents were used: 1) hexane; 2) chlorobutane/acetic acid, 100/0.1, 3) chlorobutane/acetic acid, 100/0.1; 4) ethyl acetate/acetic acid, 100/0.1; 5) acetonitrile/acetic acid, 100/0.1; 6) isopropanol/acetic acid, 100/0.1; 7) isopropanol/methanol/acetic acid, 95/5/0.1; 8) isopropanol:methanol:acetic acid, 90:10:0.1; 9) methanol/water/acetic acid, 95/5/0.1; 10) methanol/acetic acid, 95/5. From the second silica SPE eluates, fraction 4 and combined fractions 5-8 were concentrated to dryness (TurboVap, 40 ºC, 10 psi). These two concentrated samples were analyzed by LSC and submitted for MS analysis: FOR-A+B-M/H-C18EL-SiOH 8+9-concSiOH 4 and FOR-A+B-M/H-C18EL-SiOH 8+9-concSiOH 5-8.

3.9. Instrumental Methods

3.9.1. Oxidative Combustion

Determination of TRR levels is detailed in Appendix B. The amount of total ^{14}C activity in other samples, particularly post-extracted tissue, was determined by combusting aliquots 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 a Packard Tri-Carb 2500TR (SN 402385, SN 402054 with chiller, SN 402384, or SN 400287, 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)

Neutral organic extracts were made basic (pH >9) using 1 N NaOH. The samples were concentrated on a Turbovap (40 ºC water bath, 10 psi nitrogen) to remove the majority of the organic solvent. After acidifying the sample with 2 N HCl (pH <2.5) and diluting with water (4 mL), the sample was mixed. The Strata-X SPE cartridges (500 mg, 8B-S100-HDG, Phenomenex Inc., Torrance, California, USA) were conditioned with methanol (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 SPE was dried for 10 seconds after the SPE had eluted (grain samples not dried). The sample vial was rinsed with water/1.0 N HCl (99/1, v/v, 5 mL), transferred to the SPE cartridge, and eluted at approx. 2 mL/min, pooling with the load eluate. The SPE cartridge was dried under full vacuum for 20 seconds. The Strata-X SPE was eluted with methanol/water/1.0 N NaOH (80/20/1, v/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 water/methanol/acetic acid (80/20/1, 1.0 mL), and mixed well. Triplicate aliquots of each load and reconstituted elution sample were analyzed by LSC. The concentrated elution sample was also analyzed by HPLC.

3.9.4. C_{18} Solid Phase Extraction (SPE)

Neutral organic extracts were made basic (pH >9) using 1 N NaOH. The samples were concentrated on a Turbovap (40 ºC water bath, 10 psi nitrogen) to remove the majority of the organic solvent. After acidifying the sample with 2 N HCl (pH <2.5) and diluting with water (4 mL), the sample was mixed. The C_{18} SPE cartridges (500 mg, Waters Corp.) were conditioned with methanol (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/1.0 N HCl (99:1, v:v, 5 mL), transferred to the SPE cartridge, and eluted at approx. 2 mL/min, pooling with the load eluate. The SPE cartridge was dried under full vacuum for 20 seconds. The SPE was eluted with methanol/water/1.0 N NaOH (80/20/1, v/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 typically were reconstituted in 250 µL of acetonitrile, sonicated, and diluted with water (750 µL), and mixed well. Triplicate aliquots of each load and reconstituted elution sample were analyzed by LSC. The concentrated elution sample was also analyzed by HPLC.

3.9.5. 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, a 1200 Series variable wavelength detector, and a 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). A direct spike of each sample analyzed by HPLC was compared to the sum of the radioactivity eluted from the column and used to determine chromatographic recovery.

Typical retention times for 2,4-D and metabolites are shown in Table 5. A typical UV chromatogram showing the retention times for 2,4-D and reference standards used in this study is provided in Figure 3.

3.9.6. Liquid Chromatography with Mass Spectral Detection (LC/MS)

The LC/MS instrumentation and conditions are described in the mass spectral report, Appendix D. In general, analysis used liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI). A Berthold radioactivity monitor (RAM) was used to assist in location of the metabolite peaks.

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 (*7*).

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

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

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

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

Example: For the combustions, background was typically 30 dpm (grain and cobs) or 50 dpm (forage and fodder), typical aliquot weight was 0.2 g, and count time was 5 minutes).

$$\text{LOD, tissue}_{(\text{dpm})} = \frac{2.71 + \left(4.65\sqrt{30\ \text{dpm} \times 5\ \text{min}}\right)}{5\ \text{min}} = 12\ \text{dpm over background}$$

$$\text{LOD, tissue}_{(\text{ppm})} = \frac{12\ \text{dpm}}{0.2\ \text{g} \times 25{,}021\ \text{dpm/µg}} = 0.0024\ \text{µg/g}$$

$$\text{LOQ, tissue}_{(\text{dpm})} = \frac{50 \times \left(1 + \sqrt{1 + \left(\frac{30\ \text{dpm} \times 5\ \text{min}}{12.5}\right)}\right)}{5\ \text{min}} = 46\ \text{dpm over background}$$

$$\text{LOQ, tissue}_{(\text{ppm})} = \frac{46\ \text{dpm}}{0.2\ \text{g} \times 25{,}021\ \text{dpm/µg}} = 0.0092\ \text{µg/g}$$

For HPLC analyses, background was typically 8, 10, or 15 dpm, resulting in an LOD of 24.02, 26.22, or 30.89 dpm, respectively. The ARC Data System software automatically picked peaks using these values. The background value was manually adjusted from 8-15 dpm, as necessary, due to the amount of radioactivity injected and the number of peaks.

3.10.2. Statistical Methods

Statistical analyses included calculations of means, standard deviations, and Q-tests 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 analyzed by extraction.

3.10.5. Reference Values

HPLC reference values for 2,4-D and metabolites by may be found in Table 5.

4.0 RESULTS AND DISCUSSION

4.1. In-Life Summary

The average radiochemical purity of the test substance prior to application was determined to be 99.7% for the ^{14}C-2,4-D DMA, by HPLC (Figure 4). The specific activity was calculated to be 25,021 dpm/µg (3.00 mCi/mmol) for the applied ^{14}C-2,4-D DMA.

Each application solution contained 82-95% of the target amount of formulated ^{14}C-2,4-D DMA (Table 6). Overall, the plot received 492.3 mg 2,4-D DMA, equivalent to 2934 g a. e./ha or 87.3% of the target. The applications were made at an approximate 1X seasonal rate.

The average radiochemical purity (^{14}C-2,4-D) of the formulated application solutions post-application was 97.6% (93.7-99.8%). Pre-application retainer sample analyses were similar, with an average of 97.9% ^{14}C-2,4-D (93.5-100%), indicating stability of the test substance during storage and application. An example chromatogram, a post-retainer sample from the third application, is 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 Radioactive Residue

Table 7 presents the distribution of the total tissue residues within the crop harvests. Immature forage contained 3.531 mg a.e./kg. Mature grain and cobs contained 0.040 and 0.026 mg a.e./kg, respectively. Mature fodder contained 5.563 mg a.e./kg. Therefore, very little 2,4-D translocated into the grain and cobs.

Sample weights are provided in the in-life report (Appendix A).

4.3. Characterization and Identification of Residues

4.3.1. Neutral Organic Extraction (EX1)

The residue was initially extracted with neutral organic solvent (90:10 methanol:water) as described in Section 3.8.1 summarized in Table 8. Approximately 90% of the TRR (3.2 mg a.e./kg) was extracted from the immature forage plants using this procedure. Lower levels of radioactivity were extracted from the mature fodder, approximately (80% of the TRR, 4.4 mg a.e./kg). The neutral extraction removed approximately 25-30% (0.010-0.012 mg a.e./kg) and 33% of the TRR (0.009 mg a.e./kg) from mature grain and cobs, respectively.

An aliquot of each neutral organic extract was prepared for HPLC as described in Section 3.8.1. SPE recoveries were 91.9-97.9% in the eluents of the forage and fodder samples. Concentration recoveries were 54.6 and 59.9% for the grain and 72.5 and 115% for the cobs. The low grain concentration recoveries were presumably due to the high oil content. When the grain was first extracted with hexane to remove oils, SPE results showed 37-48% of the radioactivity was not retained on the SPE (9-12% of the TRR, 0.004-0.005 mg a.e./kg). The remainder of the radioactivity could be eluted off of the SPE but only a portion was able to be analyzed by HPLC (7-12% of the TRR, 0.003-0.005 mg a.e./kg); the balance of the radioactivity required stronger elution solvents and ultimately plugged the HPLC guard column.

HPLC results are summarized in Table 9. The neutral organic extract of immature forage contained primarily parent 2,4-D, as shown in Figure 8. Low levels of the dichlorophenol (DCP), 1% of the TRR, were present. The disaccharide and glucose conjugates of DCP were also present at 12% and 4% of the TRR, respectively. No other single component accounted for more than 4% of the chromatogram (≤3.0% TRR).

The neutral organic extract of fodder showed a similar HPLC profile to the forage extract, as shown in Figure 9. The major components of the fodder extract were 2,4-D (43% of the TRR), the disaccharide and glucose conjugates of DCP (8.8% and 13% of the TRR, respectively). DCP was present at very low levels (1.0% of the TRR).

The neutral organic extracts of grain were difficult to analyze by HPLC, due to low concentration/purification recovery, high pressure shutdown of the HPLC, and separation into two phases. Approximately 20% of the TRR (0.008 mg a.e./kg) was analyzed by HPLC, shown in Figure 10 and Figure 11. Parent 2,4-D and component(s) eluting at the solvent front were the only peaks present, each at levels approximately 7% of the TRR and 0.003 mg a.e./kg. The cob extracts, Figure 12, were similarly difficult to analyze, and were shown to contain only polar, solvent-front components.

HPLC results are summarized in Table 9. The neutral organic extracts of forage and fodder consisted of multiple components, including 2,4-D, glucose conjugates, and DCP. The low radioactive levels in grain were comprised of only parent and polar unknowns.

4.3.2. Methanolic Base Extraction (EX2)

The pellet remaining after the neutral extraction was next extracted with 90/10 methanol/1.0 N NaOH, as described in Sections 3.8.2 and summarized in Table 8. From the immature forage, 8.0% of the TRR was extracted with this procedure. From the mature fodder, an additional 13.5% of the radioactive residue was extracted. From mature grain and cobs, and additional 3-9% of the TRR was extracted with methanolic base, however, due to the low overall levels (<0.005 mg a.e./kg) these extracts were not analyzed by HPLC.

Following C_{18} SPE clean-up of the methanolic base extracts, the recovery in the eluates were good (95-100%); SPE recovery values were not used in the calculation of the percentage and mg/kg levels of each metabolite in the extract.

HPLC analysis of the extract of the forage and fodder showed primarily 2,4-D and low level components including the glucose conjugates of DCP, and DCP, as shown in Figure 13 (forage) and Figure 14 (fodder). HPLC results are summarized in Table 9.

4.3.3. Extraction Summary

Table 8 and Table 9 summarize the amount of the TRR that was extractable and the HPLC results, respectively. Approximately 90% of the forage and fodder TRR was extracted and analyzed by HPLC. Less than 40% of the grain and cob TRR was extracted and only approximately 20% of the TRR in these tissues was analyzed by HPLC.

Following three applications of 2,4-D DMA to AAD-1 corn, the primary residue in the forage, fodder, and grain was 2,4-D. Forage and fodder contained 4-15% of the TRR as the disaccharide of DCP and the glucose conjugate of the DCP, and very low levels of DCP (<1.5% of the TRR). Overall, 90% of the forage TRR and 80% of the fodder TRR was characterized.

4.3.4. Bound Residues

As shown in Table 8 the amount of non-extractable, or bound radioactivity, exceeded 60% of the TRR in grain and cobs. The bound residues were evaluated as described in Section 3.8.4, and the results are shown in Table 10. In the grain, the majority of the non-extractable residue was associated with the ADF soluble fraction (35.2% of the TRR, 0.014 mg a.e./kg), consisting of primarily hemicellulose and dissolved plant proteins, and lignin (14.0% of the TRR, 0.006 mg a.e./kg). In the forage and fodder, the non-extractable radioactivity was associated with pectin (3.2% and 5.1% of the TRR, respectively) and lignin (2.3% of the TRR for both).

Table 11 shows the results of the starch isolation. Overall recoveries were slightly high, 120-131%. When fresh tissue that had not been previously extracted was analyzed, the majority of

the radioactivity was extracted with DMSO but not precipitated as starch (53% of the TRR), while 31% of the TRR precipitated as starch (normalized to 100% recovery). When post-extracted grain was analyzed, approximately equal amounts of radioactivity were isolated in each of the three compartments (25-27% of the TRR, Table 11). These results indicate that a significant portion of the radioactivity, 25-40% of the TRR in grain, is closely associated with starch.

4.4. Degradate and Metabolite Isolation and Identification

Metabolites were isolated from the forage tissue, as described in Section 3.8.6. These two samples were submitted LC/MS/MS analyses. In addition to parent 2,4-D, two glucose conjugates of DCP were detected, as described in the mass spectral report, Appendix D. The glucose conjugate of DCP was observed as the formate adduct (MW 370) and was observed in both AAD-1 corn and AAD-12 soybeans (*8*). The disaccharide conjugate of DCP (also observed as a formate adduct, MW 532) contains two glycosides. The mass spectral data is provided in Appendix D.

4.5. 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 about seven weeks, as shown in Table 3. Repeat analyses of rinses and extracts stored frozen showed results similar to the initial analyses, demonstrating stability of the extracts under these storage conditions.

4.6. Metabolic Pathway

The proposed metabolic pathway is presented in Figure 15 and metabolite structures are provided in Table 12. As shown in the diagram, the metabolism of 2,4-D DMA in AAD-1 transformed maize proceeds rapidly through dissociation to the acid. After cleavage of the acid

side chain, the resulting dichlorophenol (DCP) is rapidly conjugated with glucose. Very low levels of free DCP compared to conjugated indicate that the conjugation is rapid and a preferential route of metabolism. Metabolism proceeds through natural incorporation of the radiolabeled carbon into natural plant constituents, such as pectin, lignin, and starch.

Plant metabolism studies conducted on similarly transformed AAD-12 soybeans (*8*) also concluded that metabolism proceeds to DCP and glucose conjugates of DCP.

Overall, the ^{14}C-residues in the AAD-1 transformed maize were similar to those observed on transformed soybeans and conventional crops.

5.0 CONCLUSIONS

Three applications of 2,4-D DMA salt at approximately the maximum proposed seasonal application rate resulted in immature forage and mature grain, cobs, and fodder that contained 3.531, 0.040, 0.026, and 5.563 mg acid equivalents per kg, respectively.

In the mature grain, the radioactive residue was identified as parent (7% of the TRR, 0.003 mg a.e./kg). The major metabolite in forage and fodder was also parent, 67.5% and 51.3% of the TRR (2.383 mg a.e./kg and 2.855 mg a.e./kg), respectively. Glucose-conjugated DCP was identified in forage. Approximately 30% of the TRR in grain (0.012 mg a.e./kg) was associated with starch. Extensive metabolism of 2,4-D was demonstrated by incorporation or encapsulation of radioactivity into natural plant constituents such as pectin, lignin, and starch.

In summary, the majority of the radioactive residue was characterized as 2,4-D and free or conjugated DCP.

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. 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.

3. 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.

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

5. 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.

6. 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.

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

8. Graper, L. K., Smith, K. P., Adelfinskaya, Y. A., and Balcer, J. L. A Nature of the Residue Study with [^{14}C]-2,4D DMA Applied to AAD-12 Soybeans. Unpublished report of Dow AgroSciences, study ID 090051, in-progress.

Table 1. Physico-chemical properties of 2,4-D DMA Salt

Parameter	Values	Comments
Water solubility	@ 25 ºC	
pH 5	321 g/L	Reference 1
pH 7	729 g/L	Reference 1
pH 9	664 g/L	Reference 1
unbuffered		
Vapor Pressure	$<1 \times 10^{-7}$ mm Hg @ 25 ºC	Reference 2
pK_a	acid: 2.87	Reference 3
Log K_{ow}		
Unbuffered	N/A	
pH 5	acid: 0.04	Reference 4
pH 7	acid: -0.91	Reference 4
pH 9	acid: -1.04	Reference 4
Stability of Compound at Room Temperature		
Organic Solvent Solubilities		
Acetone	N/A	
Acetonitrile	1.06 g/100 mL	Reference 5
Methanol	>50 g/100 g	Reference 5
Octanol	5.41 g/100 mL	Reference 5
Toluene	0.165 g/100 mL	Reference 5
Hexane	0.00357 g/100 mL	Reference 5

N/A = not available

[1] Hopkins, D. L. 2,4-Dichlorophenoxyacetic Acid Dimethylamine Salt: Determination of the Water Solubility. Unpublished report of Dow AgroSciences, study ID ES-DR-0008-3556-3, 1 December 1987.

[2] McDaniel, R. L. and Weiler, D. W. Vapor Pressure Determination of 2,4-Dichlorophenoxyacetic Acid Dimethylamine Salt. Unpublished report of Dow AgroSciences, study ID 41023, 30 September 1987.

[3] Martin, E. and Reading, T. J. 2,4,D; (2,4,-Dichlorophenoxyacetic acid) Dissociation Constant Determination. Unpublished report of Dow AgroSciences GH-C 1974, 25 November 1987.

[4] Bailey, R. E. and Hopkins, D. L. 2,4-Dichloropehnoxyacetic acid: Determination of Octanol/Water Partition Coefficient. Unpublished report of Dow AgroSciences, study ID ES-DR-0002-2297-9, 14 December 1987.

[5] Kinnunen, C. Determination of Solubility of 2,4-Dichlorophenoxyacetic Acid, Dimethyl Salt. Unpublished report of Dow AgroSciences, study ID FOR94078, 26 August 1994.

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	soluble liquid
Application timing and target rate (crop stage)	1121 g ae/ha – pre-emergent 1121 g ae/ha – V4 1121 g ae/ha – V8 or 48 inches, whichever comes first
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
Pre-emergent Application	29 May 2008	0	Not Applicable
Foliar Application #1 (V4)	26 June 2008	28	Not Applicable
Foliar Application #2 (V8)	08 July 2008	40	Not Applicable
Immature Harvest – Forage (R4)	07 August 2008	70	Not Applicable
Milling Completed	19 August 2008	Not Applicable	12
Combustion Analysis	21 August 2008	Not Applicable	14
Initiate Extraction	8 September 2008	Not Applicable	32
HPLC characterization init.	17 September 2008	Not Applicable	41
HPLC charact. complete	06 November 2008	Not Applicable	91
Mass Spectral Analysis initial	12 December 2008	Not Applicable	127
Mass Spectral Analysis final	07 January 2010	Not Applicable	518
Mature Harvest - Grain	03 September 2008	97	Not Applicable
Milling Completed	22 September 2008	Not Applicable	19
Combustion Analysis	30 September 2008	Not Applicable	27
Initiate Extraction	03 October 2008	Not Applicable	30
HPLC characterization init.	23 October 2008	Not Applicable	50
HPLC charact. complete	06 November 2008	Not Applicable	64
Mature Harvest - Cobs	03 September 2008	97	Not Applicable
Milling Completed	22 September 2008	Not Applicable	19
Combustion Analysis	30 September 2008	Not Applicable	27
Initiate Extraction	03 October 2008	Not Applicable	30
HPLC characterization	14 October 2008	Not Applicable	41
Mature Harvest - Fodder	03 September 2008	97	Not Applicable
Milling Completed	02 October 2008	Not Applicable	29
Combustion Analysis	06 October 2008	Not Applicable	33
Initiate Extraction	10 October 2008	Not Applicable	37
HPLC characterization init.	14 October 2008	Not Applicable	41
HPLC charact. complete	06 November 2008	Not Applicable	64

Table 4. Typical Sample Collection and Analysis Procedure for All Tissue Samples Except Where Noted (See Also Figure 5)

Parameter		Description
Neutral Extraction (EX1) *	Solvent	Methanol/water (90/10, v/v)
	Procedure*	Weigh approximately 10 or 20 g milled tissue* Add extraction solvent (approx. 50 or 100 mL) Polytron homogenize (~5 min @ ≥10K rpm) Shake on horizontal shaker (low speed) ~30 min Vacuum filter Repeat extraction 2 more times, pooling extract Record volume of the extract Transfer tissue back into original jar
	Method of analyses	LSC of triplicate aliquots of the extract
Methanolic Base Extraction (EX2)	Solvent	Methanol/1.0 N NaOH (90/10, v/v)
	Procedure	Add extraction solvent (approx. 25 or 50 mL) Heat to approximately 50 ºC for 1 hour Shake on horizontal shaker (low speed) ~60 min Vacuum filter 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

*Extracted approximately 10 g of forage or fodder tissue, or 20 g of grain or cob tissue.

Table 4, Cont. Typical Sample Collection and Analysis Procedure for All Tissue 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 3
		Sonicate or Polytron homogenize 2 minutes Heat (approx. 70 ºC) & stir approx. 5 hours Cool Vacuum filter, determine volume
	Method of analyses	LSC of triplicate aliquots of the filtrate Continue analysis of tissue with Lignin Extraction
Lignin Extraction	Solvent	Sodium hypochlorite
	Procedure	Reference 4
		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 approx. 70 ºC for 1 hour Add additional sodium chlorite (0.4 g) and glacial acetic acid (150 µL), and mix well Heat to approx. 70 ºC for 1 hour Vacuum filter Wash solids several times with water
	Method of analyses	LSC of triplicate aliquots of the filtrate 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 5
		Reflux 1 hour Vacuum filter Rinse solids with water and acetone Determine volume of filtrate
	Method of analyses	LSC of triplicate aliquots of the filtrate Combust ADF

Table 5. HPLC Conditions

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	280 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	
20.0	50/50 A/B, linear gradient	
30.0	5/95 A/B, begin 10 minute hold	
40.0	5/95 A/B, linear gradient to original conditions	
41.0	90/10 A/B, begin re-equilibration	
50.0	90/10 A/B, end run	

Compound	HPLC Retention Time (min)[a]
4OH 2,5-D	16.5
4-CPAA	20.6
4-CP	22.2
2,4-D	24.3
phenol	25.9

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

Table 6. Application Rate of 2,4-D DMA Applied to AAD-1 Corn

Application	Actual (dpm/0.10 mL)	Volume (mL)[a]	Amount Applied (mg a.i.)[b]	Application Rate (g a.e./ha)[c]	Target Application Rate (g a.e./ha)[d]	% of Target
1 - pre-emergent	2,606,867	148.7	154.9	923	1121	82.4
2 - foliar	2,649,329	148.7	157.5	938	1121	83.7
3 - foliar	2,265,402	198.7	179.9	1072	1121	95.7
Total Applied (Seasonal):			492.3	2934	3363	87.3

[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 25,021 dpm/µg.

[c] Application rate = $\frac{\text{Amount Applied (mg)}}{1000\ \text{mg/g} \times \text{plot size (ha)}} \times 0.831$, where plot size was 1.39×10^{-4} ha and factor 0.831 converted from a.i. to a.e. (see sample calculations) (rounding differences noted).

[d] Target does not include any overages.

Table 7. Total Radioactive Residues in Plant Samples Collected for 2,4-D DMA Nature of Residue in AAD-1 Corn Study

Sample	dpm/g	mg a.e./kg (ppm)
forage (immature plants)	106,360	3.531
mature grain	1219	0.040
mature cobs	777	0.026
mature fodder	167,579	5.563

Table 8. Fractionation of the Residues in 2,4-D Treated AAD-1 Corn RACs (Average of Duplicates)

	TRR[a]	Neutral Organic Extract		Basic Methanol Extract		Non-Extractable		Total Recovered	
Sample ID	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg
Forage	3.531	90.4	3.192	8.0	0.284	8.6	0.303	107	3.779
Grain (A&B)	0.040	29.1	0.012	8.3	0.003	64.2	0.026	102	0.041
Grain (C&D)	0.040	24.5[b]	0.010[b]	8.8	0.004	67.7	0.027	103[b]	0.042
Cobs	0.026	33.4	0.009	3.3	<0.001	66.5	0.017	103	0.027
Fodder	5.563	79.6	4.429	13.5	0.752	10.1	0.564	103	5.745

NA = Not applicable

[a] From Table 7.

[b] Grain replicates C & D were each first extracted with hexane, followed by the neutral organic extract. The hexane removed an average of 2.3% of the TRR, <0.001 mg ae/kg.

Table 9. 2,4-D and Metabolite Levels (Acid Equivalents) In Extracts of 2,4-D Treated AAD-1 Corn RACs (Average of Duplicates)

Sample ID	2,4-D 24.5 min		disaccharide conjugate of DCP 14.2 min		glucose conjugate of DCP 17.1 min		dichlorophenol (DCP) 26 min		unidentified[a] extractable residue	
	% TRR	mg a.e./kg	% TRR	mg a.e./kg	% TRR	mg a.e./kg	% TRR	mg a.e./kg	% TRR	mg a.e./kg
Forage										
neutral extract	62.6	2.209	12.0	0.422	4.1	0.144	1.0	0.035		
base extract	4.9	0.174	0.2	0.007	0.7	0.026	0.4	0.013		
total	67.5	2.383	12.2	0.430	4.8	0.170	1.4	0.048	9.9	0.351
Grain										
neutral extract	7.1	0.003	ND	ND	ND	ND	ND	ND		
total	7.1	0.003	ND	ND	ND	ND	ND	ND	86.0	0.035
Cobs										
neutral extract	ND	ND	ND	ND	ND	ND	ND	ND		
total	ND	ND	ND	ND	ND	ND	ND	ND	77.3	0.020
Fodder										
neutral extract	43.2	2.403	8.8	0.490	12.7	0.709	1.0	0.057		
base extract	8.1	0.452	0.7	0.039	1.9	0.105	0.1	0.006		
total	51.3	2.855	9.5	0.529	14.6	0.815	1.1	0.063	20.2	1.125

ND = not detected

[a] Unidentified radioactivity did not elute with 2,4-D, the glucose conjugates of DCP, DCP, solvent-front polar material (<1.5% of the TRR in forage and fodder), or an 18.3 minute unknown metabolite (<3% of the TRR, present in forage and fodder only).

Table 10. Fractionation of the Bound Residues in 2,4-D Treated AAD-1 Corn (Average of Duplicates)

	Bound Residue[a]		Pectin		Lignin		ADF soluble		ADF		Recovery
Sample ID	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%TRR	mg a.e./kg	%[b]
Forage	8.6	0.303	3.2	0.114	2.3	0.080	0.8	0.029	0.3	0.012	77.4
Grain (A&B)	64.2	0.026	3.0	0.002	14.0[c]	0.006	35.2	0.014	1.4	<0.001	81.7
Cobs	66.5	0.017	7.6	0.002	5.0	0.001	--[d]	--	--	--	--
Fodder	10.1	0.564	5.1	0.282	2.3	0.131	0.8	0.044	0.3	0.019	84.2

[a] Values from Table 8
[b] Procedural recovery for the 4-step process.
[c] Replicate B jar broke, results for replicate A only for remainder of procedure.
[d] Samples discontinued through the remaining procedure.

Table 11. Starch Isolation from Mature Grain of AAD-1 Corn Treated with ^{14}C-2,4-DMA (Average of Duplicates)

Sample ID	Non-Extractable		Extractable (non-starch)		Starch		Recovery
	% TRR	mg a.e./kg	% TRR	mg a.e./kg	% TRR	mg a.e./kg	
Grain[a]	16.3	0.007	53.1	0.021	30.6	0.012	100%
NER[b]	25.1	0.010	25.9	0.010	26.3	0.011	120%

[a] Replicates E & F, fresh tissue. Recovery was 131% prior to normalization.
[b] Non-extractable residue remaining after methanolic base extraction, where 64.2% of the TRR was non-extractable residue for replicates A & B, see Table 8.

Table 12. Metabolites of 2,4-D Applied to AAD-1 Maize

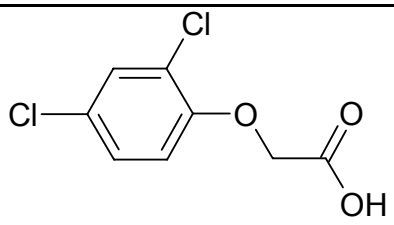
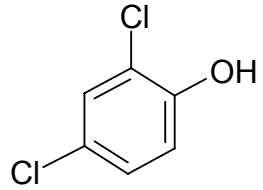
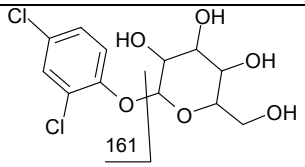
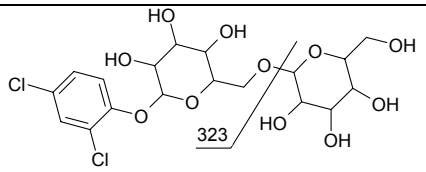
Common name/code or ID	Chemical Name	Chemical Structure
2,4-D	2,4-dichlorophenoxy acetic acid	
DCP	2,4-dichlorophenol	
X11963417, glucose conjugate of DCP	2,4-dichlorophenyl β-D-glucopyranoside	
disaccharide of DCP	2,4-dichlorophenyl 6-*O*-hexopyranosylhexopyranoside	 or isomer

Figure 1. Chemical Nomenclature and Structures of 14C-2,4-D

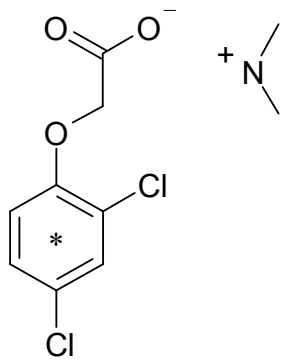
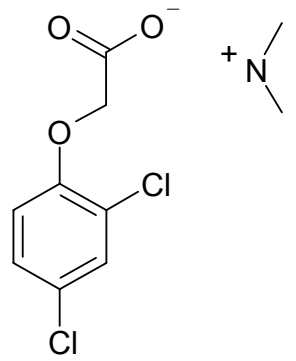
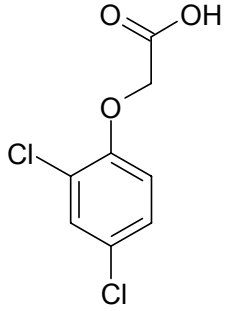
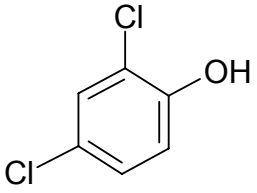
	Test Substance	Structure
Common Name	14C-2,4-D DMA salt	
Synonyms	^{14}C-2,4-D	
Chemical Name	2,4-dichlorophenoxyacetic acid-dimethylammonium salt, Phenyl ring-14C (UL)	
Inventory Number	INV027475-0001	
FA & PC Reference	FAPC-084-003	
SPS Reference	037476-40	
Specific Activity	43.4 mCi/mmol (163 µCi/mg)	
Radiochemical purity	99.2%	
GLP analysis	Yes, 3/11/2011	* denotes ^{14}C

Figure 2. Chemical Nomenclature and Structures of 2,4-D Reference Standards and Formulation Blank

	Reference Substances	Structure
Common Name	**2,4-D DMA**	
Synonyms	TSN100485	
CAS Nomenclature	acetic acid, (2,4-dichlorophenoxy)-,compd. with N-methylmethanamine (1:1)	
IUPAC Nomenclature	2,4-dichlorophenoxy acetic acid dimethylamine salt	
CAS Number	2008-39-1	
Molecular Weight	266.13 g/mole	
Molecular Formula	$C_8H_6Cl_2O_3 \cdot C_2H_8N$	
Purity	>99% (GLP)	

Common Name	**2,4-D**	
Synonyms	AGR275828	
CAS Nomenclature	(2,4-dichlorophenoxy)acetic acid	
IUPAC Nomenclature	2,4-dichlorophenoxy acetic acid	
CAS Number	94-75-7	
Molecular Weight	221.04 g/mole	
Molecular Formula	$C_8H_6Cl_2O_3$	
Purity	99.5% (GLP)	

Common Name	**2,4-dichlorophenol**	
Synonyms	AGR182992, 2,4-DCP	
CAS & IUPAC Nomenclature	2,4-dichlorophenol	
CAS Number	000120-83-2	
Molecular Weight	163.00 g/mole	
Molecular Formula	$C_6H_4Cl_2O$	
Purity	99% (GLP)	

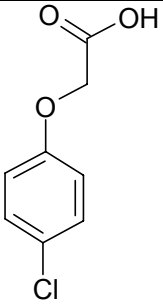
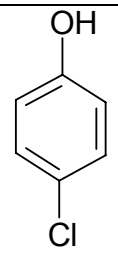
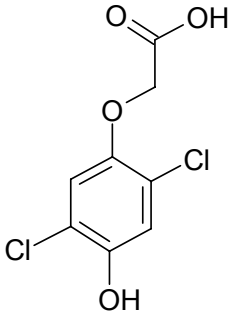
Common Name	**4-CPAA**	
Synonyms	TSN100163, 4-CPA	
CAS Nomenclature	acetic acid, (4-chlorophenoxy)-	
IUPAC Nomenclature	4-chloro-phenoxyacetic acid	
CAS Number	122-88-3	
Molecular Weight	186.60 g/mole	
Molecular Formula	$C_8H_7ClO_3$	
Purity	99% (GLP)	

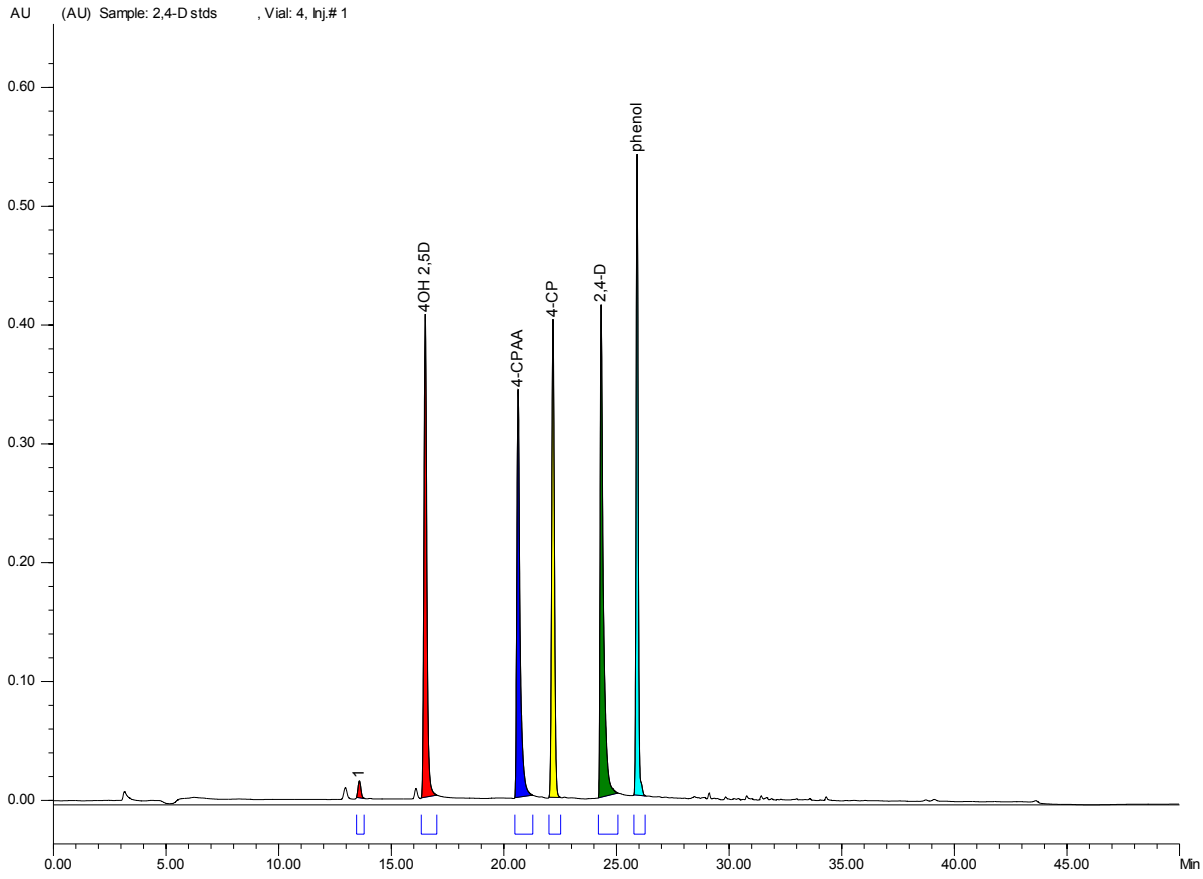
Figure 2, Cont. Chemical Nomenclature and Structures of 2,4-D Reference Standards and Control Substance

	Reference Substances	Structure
Common Name	**4-CP**	
Synonyms	TSN100174	
CAS & IUPAC Nomenclature	4-chlorophenol	
CAS Number	000106-48-9	
Molecular Weight	128.56 g/mole	
Molecular Formula	C_6H_5ClO	
Purity	99.7% (GLP)	

Common Name	**4-OH 2,5-D**	
Synonyms	TSN100130	
CAS Nomenclature	not available	
IUPAC Nomenclature	2,5-dichloro-4-hydroxyphenoxyacetic acid	
CAS Number	not available	
Molecular Weight	237.04 g/mole	
Molecular Formula	$C_8H_6Cl_2O_4$	
Purity	91.5% (GLP)	

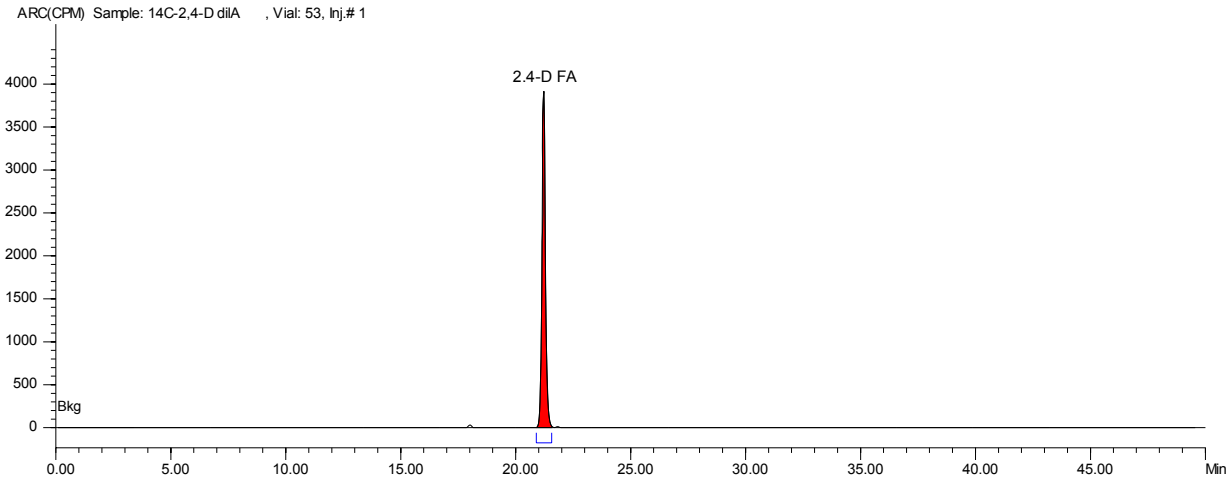
	Formulation Blank		
Common Name	F-1222-208 Formulation Blank		
Formulation Type	soluble liquid (SL)		
Description	Component	Technical Wt%	Batch (g)
	dimethylamine (DMA)	5.00	11.04
	Polyglycol P-4000	0.10	0.22
	Versene Acid (EDTA)	3.00	6.62
	Water	45.34	100.11
	2,4-D (added later)	46.56	
	total	100.00	

Figure 3. Chromatograms of Test and Reference Substances with retention times clearly indicated for each substance



Summary Table	
Retention Time (min)	Reference
16.5	4OH 2,5-D
20.6	4-CPAA
22.2	4-CP
24.3	2,4-D
25.9	phenol

Figure 4. Radio-chromatogram of ^{14}C-XDE-2,4-D Test Substance (Dilution A)



Summary Table		
Retention Time (min)	% of HPLC	Reference
21.2	99.5	2,4-D (free acid)
HPLC recovery 104%		

Figure 5. Schematic Flowchart for the Analysis of Crop Fractions (See Also Table 4)

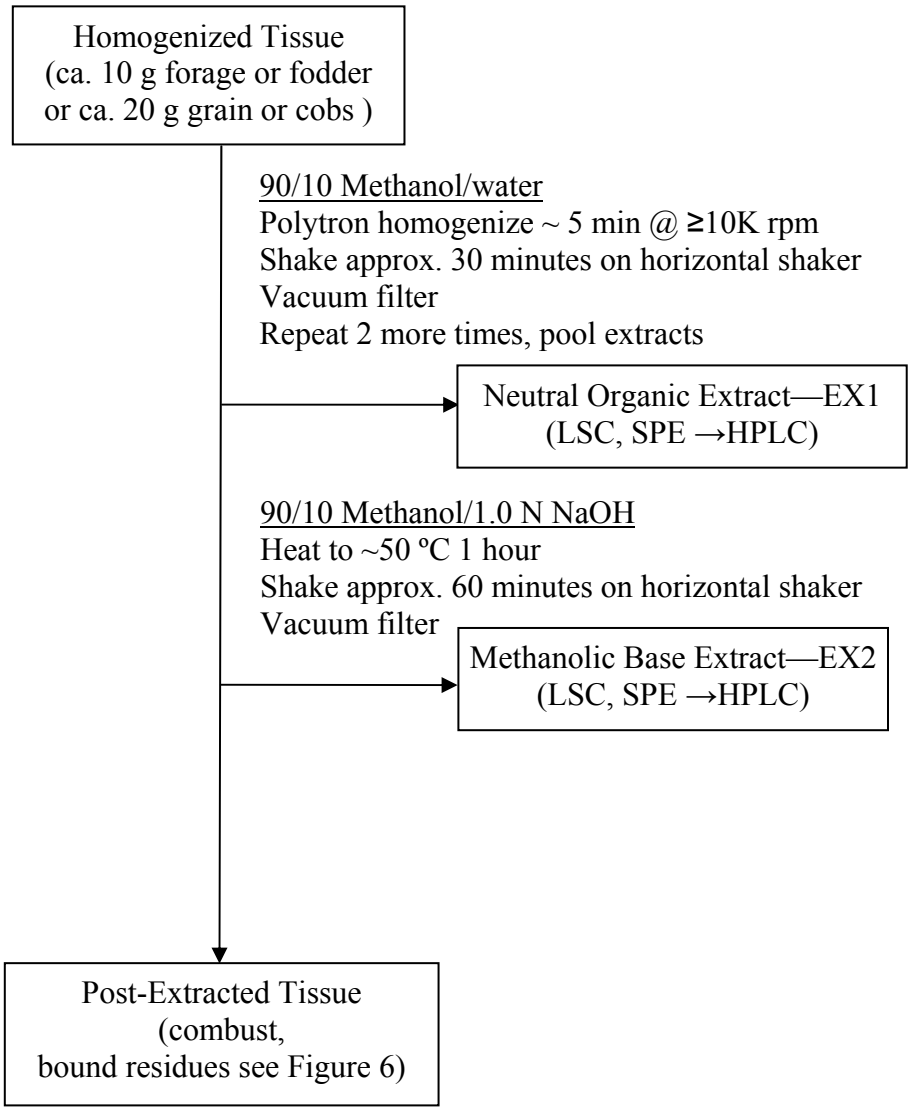


Figure 6. Schematic Flowchart for the Analysis of Bound Residues in Crop Fractions

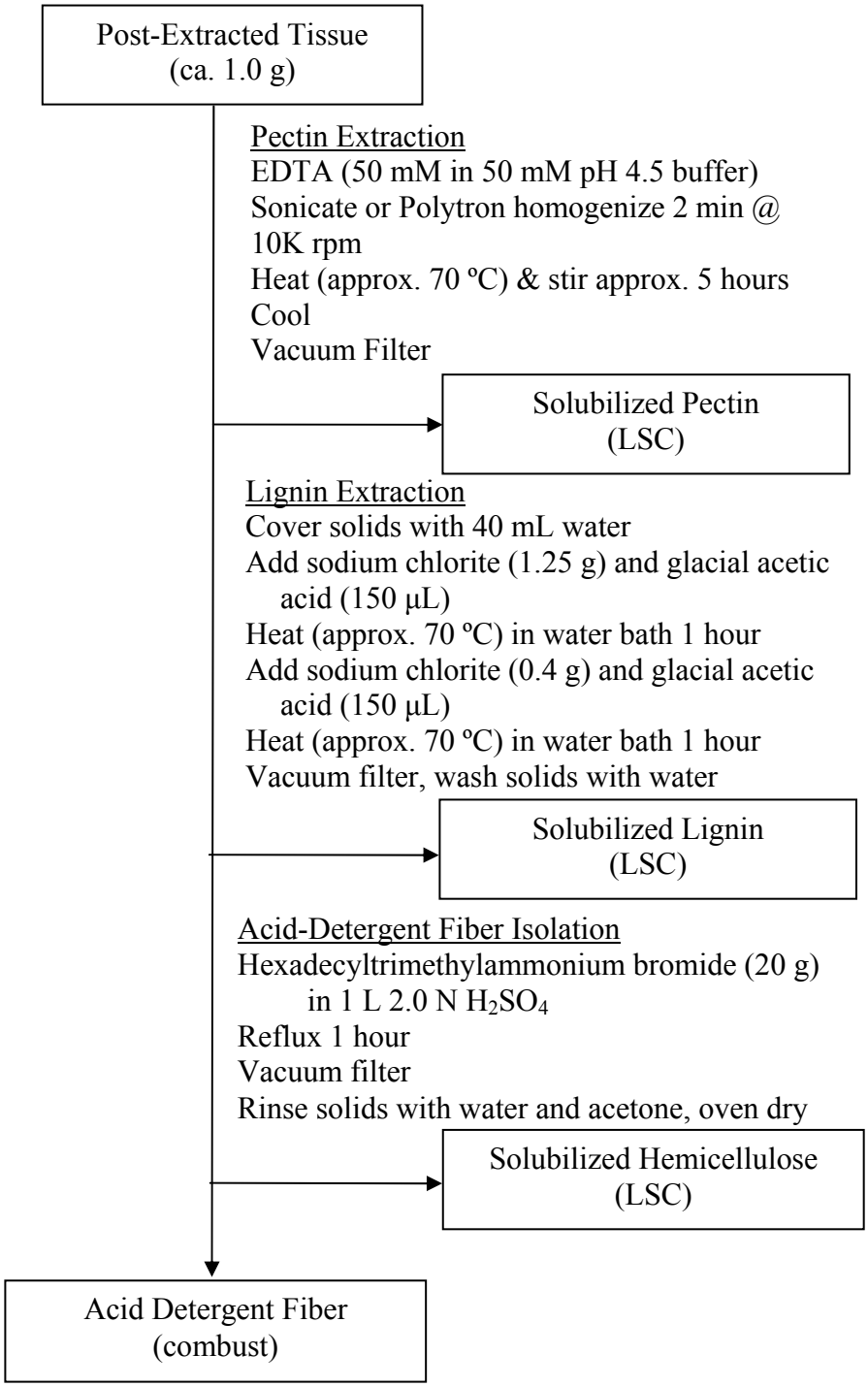
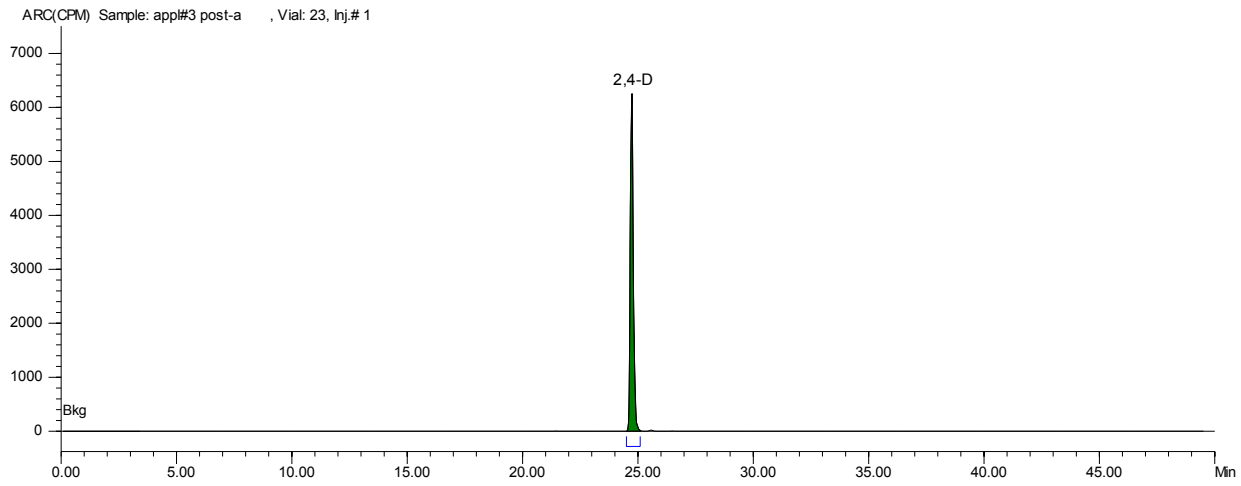
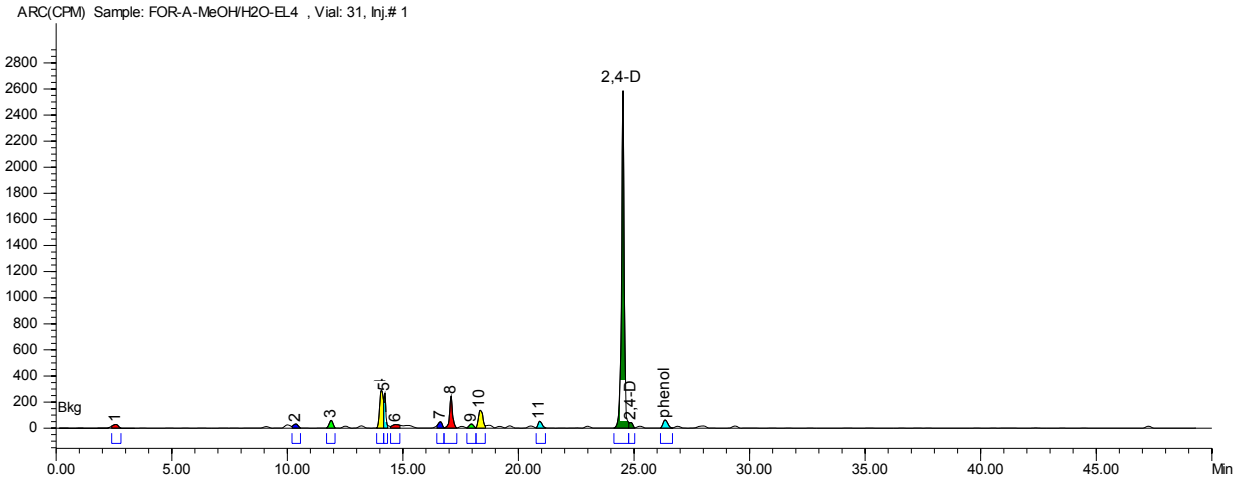


Figure 7. HPLC Chromatogram Indicating Radiochemical Purity of the ^{14}C-2,4-D DMA After the Third Application



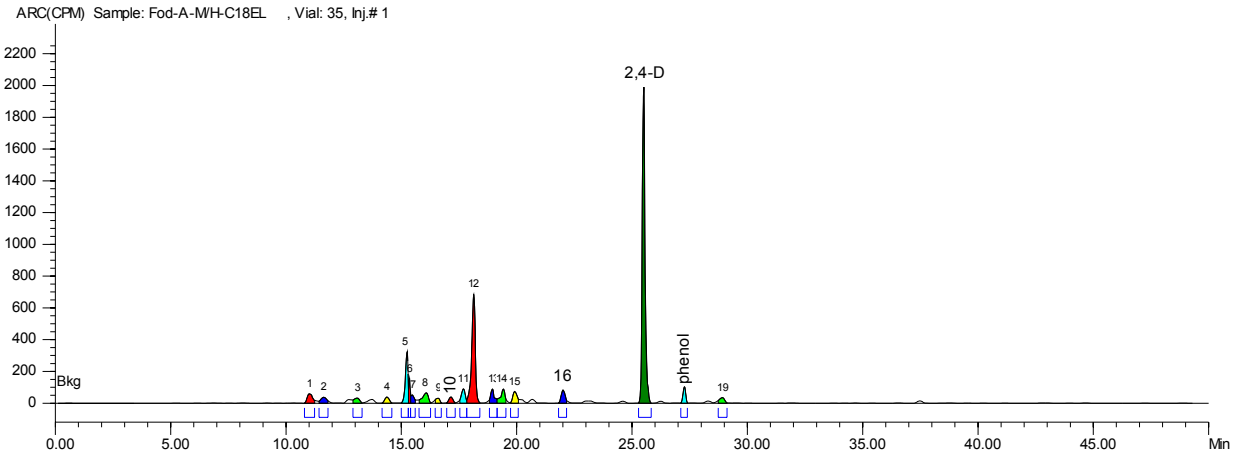
Summary Table		
Retention Time (min)	% of HPLC	Reference
24.7	99.8	2,4-D
HPLC recovery 94.9%		

Figure 8. HPLC Radio-Chromatogram of the Immature Forage – Neutral Organic Extract (Replicate A)



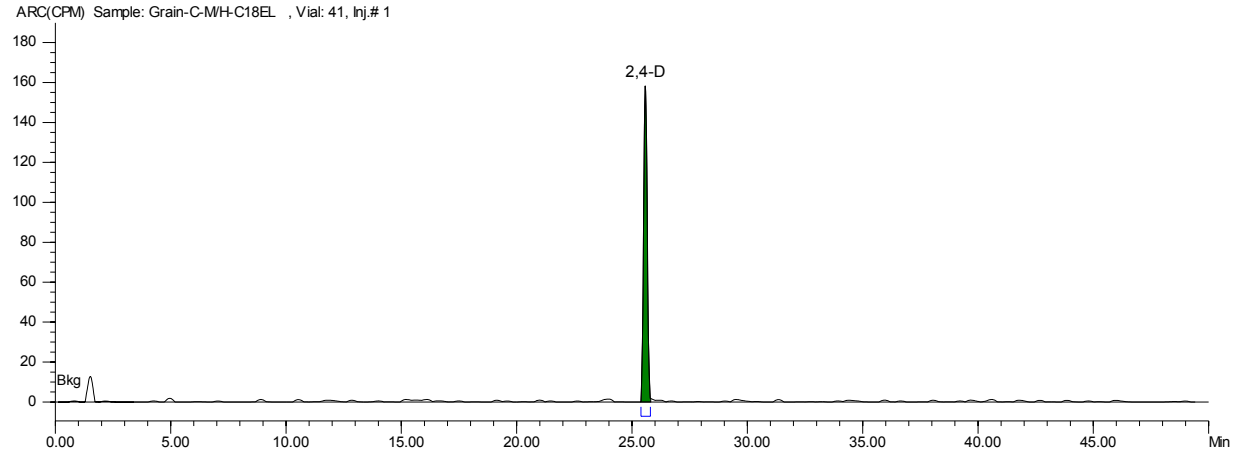
Summary Table			
Retention Time (min)	% of HPLC		Peak ID (#)
2.6	0.5		(1)
10.4	0.5		(2)
11.9	0.7		(3)
14.1	3.6	12.1	disaccharide conjugate of DCP
14.2	8.5		
14.7	0.7		(6)
16.6	0.8		(7)
17.1	5.3		glucose conjugate of DCP
18.0	0.4		(9)
18.3	3.2		(10)
20.9	1.2		(11)
24.5	69.5	70.3	2,4-D
24.9	0.7		
26.3	1.2		phenol
HPLC recovery 83.0%			

Figure 9. HPLC Radio-Chromatogram of the Fodder – Neutral Organic Extract (Replicate A)



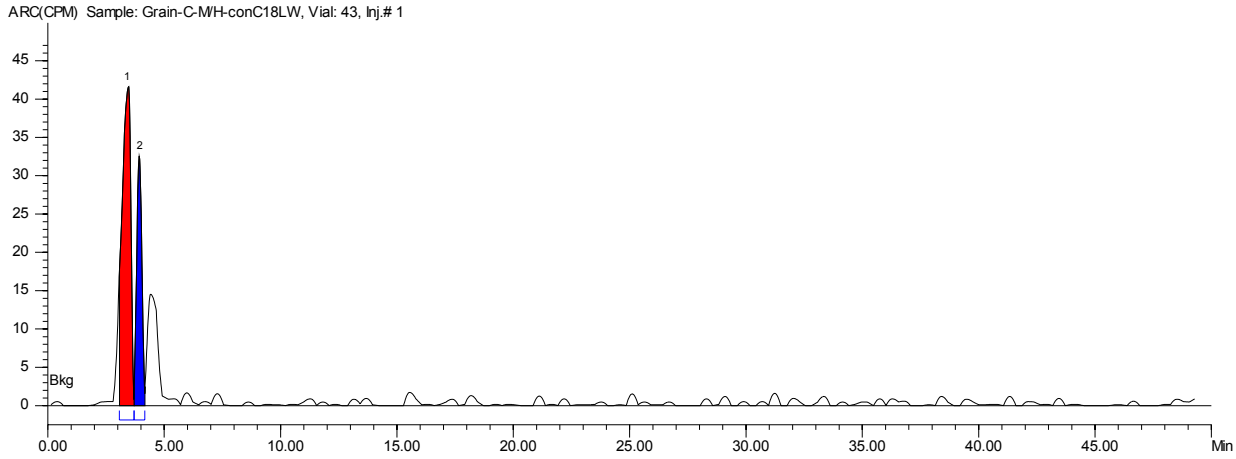
Summary Table			
Retention Time (min)	% of HPLC		Peak ID (#)
11.0	1.4		(1)
11.6	0.7		(2)
13.1	0.6		(3)
14.4	0.5		(4)
15.3	5.6	10.1	disaccharide conjugate of DCP
15.4	4.5		(5,6)
15.5	2.2		(7)
16.1	1.2		(8)
16.6	0.6		(9)
17.2	0.4		(10)
17.7	1.4		(11)
18.1	17.8		glucose conjugate of DCP (12)
19.0	1.8		(13)
19.4	1.8		(14)
19.9	0.8		(15)
22.0	1.1		(16)
25.5	55.0		2,4-D
27.3	1.2		phenol
28.9	0.6		(19)
HPLC recovery 84.8%			

Figure 10. HPLC Radio-Chromatogram of the Grain – Neutral Organic Extract, C_{18} Eluent (Replicate C)



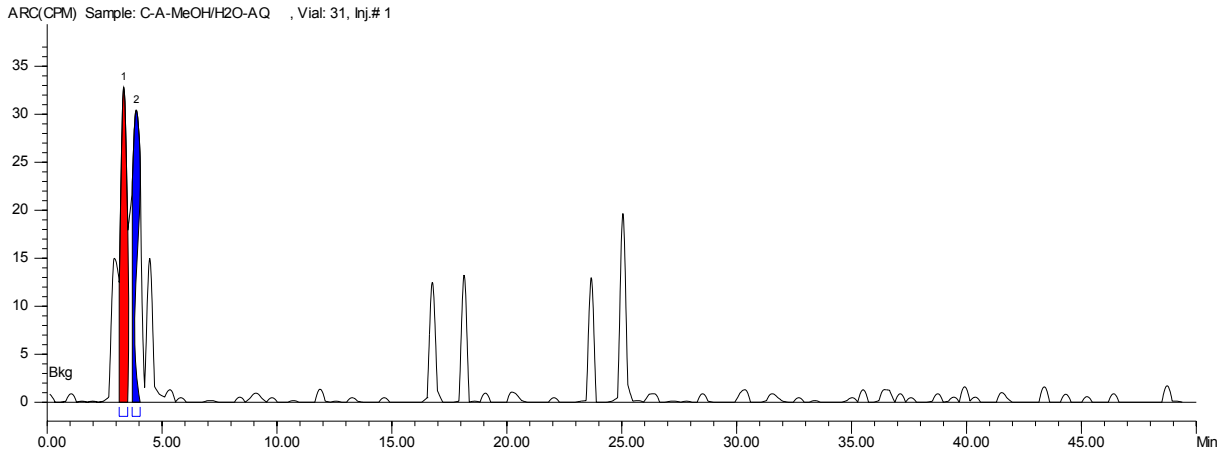
Summary Table		
Retention Time (min)	% of HPLC	Peak ID
25.6	91.2	2,4-D
HPLC recovery 88.1%		

Figure 11. HPLC Radio-Chromatogram of the Grain – Neutral Organic Extract, C_{18} Load/Wash (Replicate C)



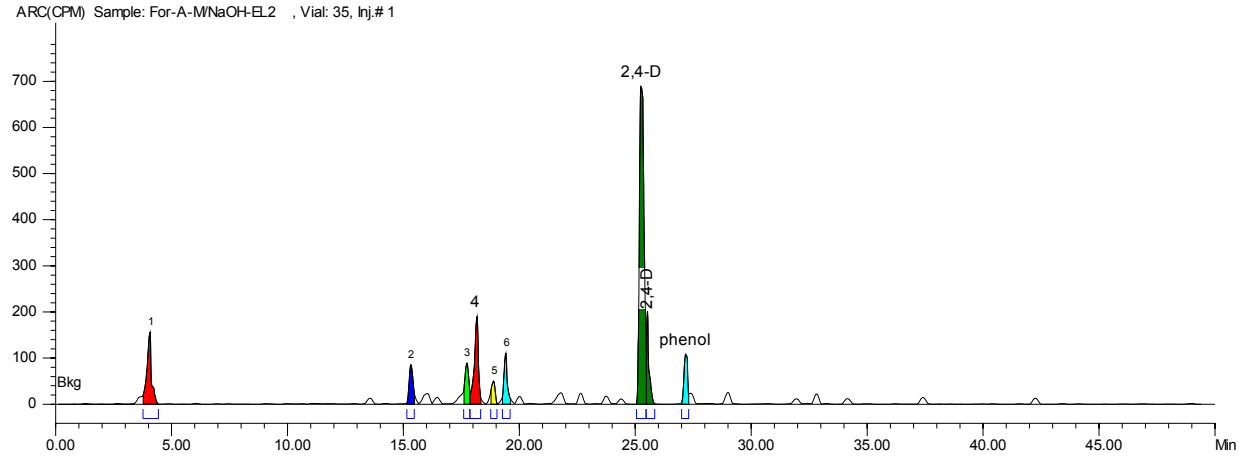
Summary Table		
Retention Time (min)	% of HPLC	Peak ID (#)
3.5	44.5	(1)
3.9	19.9	(2)
HPLC recovery 95.7%		

Figure 12. HPLC Radio-Chromatogram of the Cobs – Neutral Organic Extract, Aqueous phase (Replicate A)



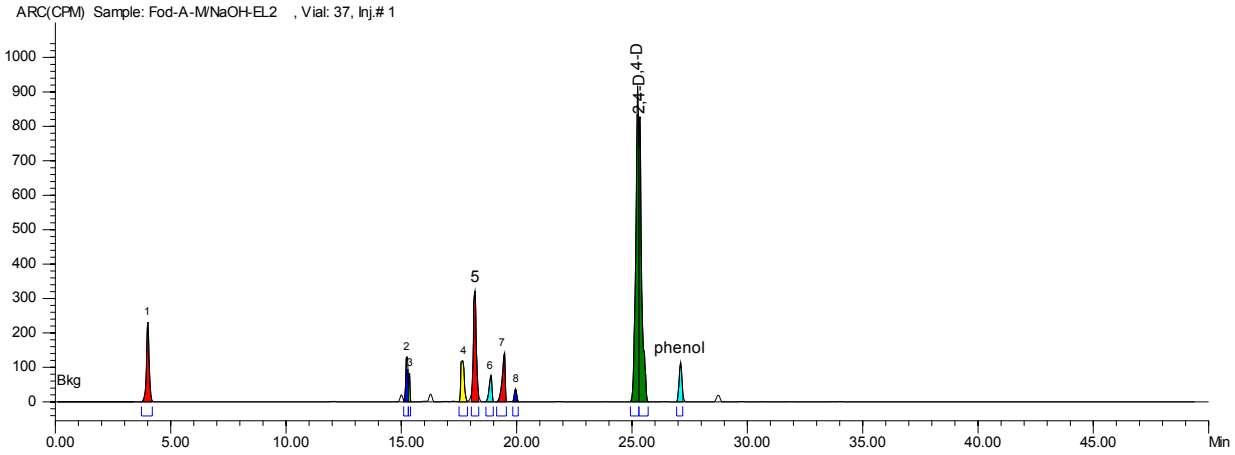
Summary Table		
Retention Time (min)	% of HPLC	Peak ID (#)
3.3	15.8	(1)
3.9	19.3	(2)
HPLC recovery 80.6%		

Figure 13. HPLC Radio-Chromatogram of the Immature Forage – Methanolic Base Extract (Replicate A)



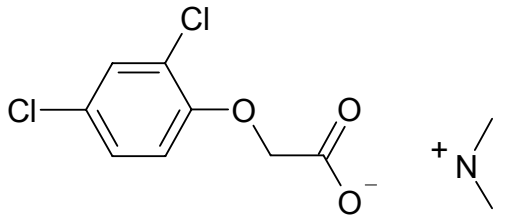
Summary Table			
Retention Time (min)	% of HPLC		Peak ID (#)
4.1	7.5		(1)
15.3	2.7		disaccharide conjugate of DCP (2)
17.7	2.8		(3)
18.2	9.0		glucose conjugate of DCP (4)
18.9	1.1		(5)
19.4	4.2		(6)
25.2	44.9		2,4-D
25.5	12.9	57.8	
27.2	5. 5		phenol
HPLC recovery 75.6%			

Figure 14. HPLC Radio-Chromatogram of the Fodder – Methanolic Base Extract (Replicate A)

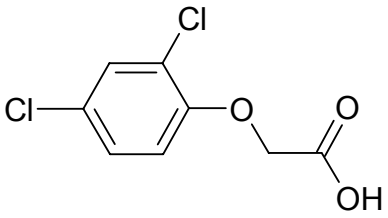


Summary Table			
Retention Time (min)	% of HPLC		Peak ID (#)
4.0	6.2		(1)
15.3	3.7		disaccharide conjugate of DCP (2)
15.4	2.6		(3)
17.7	5.0		(4)
18.2	12.8		glucose conjugate of DCP (5)
18.9	1.7		(6)
19.5	4.1		(7)
20.0	0.5		(8)
25.2 25.3	29.0 32.1	61.2	2,4-D
27.1	1.6		phenol
HPLC recovery 100%			

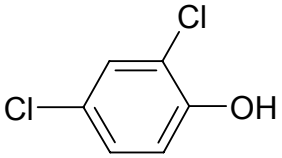
Figure 15. Proposed Metabolic Pathway for 2,4-D DMA salt in AAD-1 Maize



2,4-D DMA salt



2,4-D



dichloro-phenol (DCP)

glucose conjugates of DCP
and natural incorporation/encapsulation

Appendix A—In-Life Report

(Research For Hire)

IN-LIFE PHASE FINAL REPORT

STUDY TITLE

A Nature of the Residue Study with 2,4-D DMA salt applied to AAD-1 Maize 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 Rotondaro
Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, IN 46268

IN-LIFE PHASE COMPLETION DATE

September 3, 2008

STUDY IDENTIFICATION

Dow AgroSciences: Protocol No. 080058 Project No.10001126-5051-4
Research For Hire: Study Number R050804

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

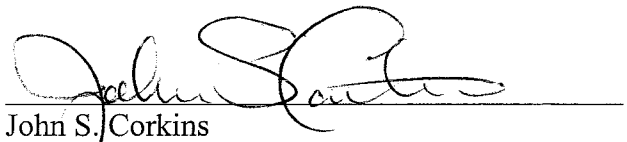
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1) (A), (B), or (C).

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

1-8-09
Date

REGULATORY COMPLIANCE STATEMENT

A Nature of the Residue Study with 2,4-D DMA salt 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
5/29/08	First Application	6/10/08
8/07/08	Forage Sampling	8/12/08
1/02/09	Raw Data Audit and In-Life Report	1/08/09

Mary Jones
Quality Assurance

4/28/10
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

1/8/09
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
Tom Sukut	Technician I
Joshua Tilton	Technician I
Stephanie Phipps	Office Coordinator

DOW AGROSCIENCES

Personnel	Position
Sandra Rotondaro	Study Director

REPORT APPROVAL

Sandra Rotondaro
Study Director
Dow AgroSciences, LLC

03 May 2010
Date

John S. Corkins
General Manager
Research For Hire

1-8-09
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 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: RFH Experimental Start Date: RFH Experimental End Date:	May 25, 2008 May 29, 2008 September 3, 2008

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

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

Page

LIST OF TABLES

Page

LIST OF APPENDICES

Page

INTRODUCTION

In this study, the radiolabeled test substance, ^{14}C-2,4-D DMA salt 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 080058. The in-life phase was contracted to Research For Hire (RFH), and the RFH study number was R050804.

OBJECTIVE

To determine the nature, amount and distribution of residues in the forage, fodder, cobs, and grain of maize plants following a pre-plant application to the soil and two applications of [^{14}C]- 2,4-D DMA salt 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 2, 4D-DMA salt applied to AAD-1 Maize 2008" (protocol no. 080058) 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 May 29, 2008 to September 3, 2008. The [^{14}C]-2,4-D DMA salt treated box was 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

Test Material

- ^{14}C- 2,4-D DMA salt

Chemical name:	2,4-dichlorophenoxy acetic acid dimethylammonium salt, Phenyl ring-14C(UL)
Common name:	2,4-D DMA salt-Phenyl-UL-14C (or 14C-2,4-D)
Lot or Identification Nos.:	INV027475-0001
Stated specific activity:	11.3 µCi
Radiopurity:	99.2%
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.

The 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. Three vials of ^{14}C-2,4-D DMA salt, containing a total of 5.706 mCi were received.

Upon receipt, 0.194 g of blank formulation (F1222-208) 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 and 8.89 °C (35°F and 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.

One planting box was used to conduct this study, 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), was double-lined with 6-mil plastic and filled with clay loam soil to within approximately 5 cm (2 inches) from the top. The one box was treated with ^{14}C-2,4-D DMA salt.

Separate control plants were not needed for this study, since they were identical to those used in DAS study 080057 (RFH study ID R050802). Therefore, the control plot for study 080057 was also used for this study, 080058.

The treated box was 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 box and was raised at the time of the test material application to prevent cross contamination. The test plot (box) was identified with a placard bearing the study number, project number, test material ID (treated), date of application, transgenic ID and the name of the Research For Hire (RFH) study coordinator.

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 the test plot (box). 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) 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 box was planted with variety pDAS 1740-474 (from DAS) on May 28, 2008. 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 box into 2 rows, with a row spacing of 24 inches, and a plant spacing of approximately 4 inches.

FERTILIZATION

The test box was 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 necessary 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 May 15, 2008.

FIRST APPLICATION

The spray solution for the first application was prepared on May 29, 2008 using the test substance labeled "080058 application solution #1". The ^{14}C-2,4-D DMA test substance was removed from the refrigerator and allowed to come to room temperature. A glass beaker with a minimum capacity of 200 mL was used to prepare the application solution. Distilled water, 150 mL, was measured and approximately half was transferred to the glass beaker reserving the other half for rinsing and the final dilution. Using a transfer pipette, the 14C-2,4-D DMA (in approximately 0.2 g formulation blank F1222-208) was quantitatively transferred from the shipping vial into the beaker and swirled to dissolve. The vial was then rinsed with ten sequential 2 mL portions of the reserved water, shaken well, and transferred to the beaker. The remaining amount of water needed to bring the

spray solution to the desired volume (150 mL) was then added. The solution was mixed on a magnetic stir plate, at medium speed, with no heat for 5 minutes.

Three (3) 0.1 mL aliquots were removed from the application solution to clean LSC vials. Exactly 9.90 mL of acetonitrile were added to each of the three aliquots, bringing their final volume to 10 mL. Each vial was capped and mixed thoroughly by inversion. Three (3) 0.10 mL 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 LSC for one minute and five minute counts.

Four (4) 0.25 mL aliquots (2 pre-aliquots and 2 post-aliquots) were taken. The pre-aliquots were placed immediately into the RFH walk-in freezer EQP 28-2 and the post-aliquots were transported to the field along with the spray solution. After application the post-application retention aliquots were stored in the RFH walk-in freezer EQP 28-2.

TIME AND RATE OF FIRST APPLICATION

The application was made on May 29, 2008 to the treated box. The spray solution was applied to the bare soil, before the corn had emerged. The spray solution was applied with a plastic bottle covered in aluminum foil (to protect against photodegradation) that was equipped with an R&D sprayer, model GS (EQP 11-4), using a single aluminum spray wand and 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. The radiolabeled treatment solution contained 150 mL of final spray solution and 148.7 mL was actually applied to the plot. The spray solution was applied evenly in two passes per row of corn. Following the application the empty spray container was rinsed with 30 mL of distilled water which was sprayed on the plot in a manner similar to the application solution.

SECOND APPLICATION

The spray solution for the second application was prepared on June 26, 2008 using the test substance labeled "080058 application solution #2". The solution was prepared in the same manner as the first application solution.

TIME AND RATE OF SECOND APPLICATION

The application was made on June 26, 2008 to the treated box. The spray solution was applied to the corn plants at theV4 growth stage. The application was conducted in the same manner as the first application.

THIRD APPLICATION

The spray solution for the third application was prepared on July 8, 2008 using the test substance labeled "080058 application solution #3". The solution was prepared in the same manner as the first application solution, with the exception that the volume of distilled water used to dilute the test substance was 200 mL.

TIME AND RATE OF THIRD APPLICATION

The application was made on July 8, 2008 to the treated box. The spray solution (198.7 mL) was applied to the corn plants at the V8 growth stage. The application was conducted in the same manner as the first application.

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 immature corn forage occurred. The corn plants were at the R4 (milky inner fluid in kernel) growth stage. Two entire plants 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 bag. After weighing on scale 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 and lab coats.

MATURE CORN 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 cobs were removed from stalks; stalks were cut approximately three inch 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 and lab coats.

SAMPLE HANDLING

Table 4 summarizes the dates of sample shipment.

SAMPLE SHIPMENTS

All treated 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
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 box.

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

Photographs were taken on the days of applications and sampling events, and stored in the raw data. Treated corn forage, fodder, and mature corn grain and cobs were harvested to enable determination of the nature of the residue of ^{14}C-2,4-D applied to AAD-1 corn.

TABLE 1 - TEST MATERIAL RECEIPT AND DISTRIBUTION

Material	Date	Distribution	Purpose	Quantity
^{14}C-2,4-D DMA	5-15-08	Receipt	Received	1.902 mCi
1st Application	5-29-08	Application	1st Application	1.902 mCi
^{14}C-2,4-D DMA	5-15-08	Receipt	Received	1.902 mCi
2nd Application	6-26-08	Application	2nd Application	1.902 mCi
^{14}C-2,4-D DMA	5-15-08	Receipt	Received	1.902 mCi
3rd Application	7-8-08	Application	3rd Application	1.902 mCi

TABLE 2: SOIL ANALYSIS RESULTS

pH	7.6
Bulk Density, disturbed (gm/cc)	1.16
Field Water Holding Capacity (% @ 1/3 bar)	23.4
Cation Exchange Capacity (meq/100 g)	27.3
Organic Matter (%) Walkley Black	3.2
Texture	47% sand-22% 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 ~5 miles Southwest.

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

Sample Number	Sample Description	Plot ID	Sample Weight (g)	Date Sampled	Date Shipped
R050804-Soil Char	Soil Characterization	Pre app 2,4-D DMA salt	NA	5/28/08	5/28/08
R050804-1	Pre-applic retention (a) applic 1	Pre-treatment	NA	5/29/08	6/2/08
R050804-2	Pre-applic retention (b) applic 1	2,4-D DMA salt	NA	5/29/08	6/2/08
R050804-3	Post-applic retention (a) applic 1	2,4-D DMA salt	NA	5/29/08	6/2/08
R050804-4	Post-applic retention (b) applic 1	2,4-D DMA salt	NA	5/29/08	6/2/08
R050804-5	Pre-applic retention (a) applic 2	2,4-D DMA salt	NA	6/26/08	6/30/08
R050804-6	Pre-applic retention (b) applic 2	2,4-D DMA salt	NA	6/26/08	6/30/08
R050804-7	Post-applic retention (a) applic 2	2,4-D DMA salt	NA	6/26/08	6/30/08
R050804-8	Post-applic retention (b) applic 2	2,4-D DMA salt	NA	6/26/08	6/30/08
R050804-9	Pre-applic retention (a) applic 3	2,4-D DMA salt	NA	7/8/08	7/14/08
R050804-10	Pre-applic retention (b) applic 3	2,4-D DMA salt	NA	7/8/08	7/14/08
R050804-11	Post-applic retention (a) applic 3	2,4-D DMA salt	NA	7/8/08	7/14/08
R050804-12	Post-applic retention (b) applic 3	2,4-D DMA salt	NA	7/8/08	7/14/08
R050804-13	Forage plants	2,4-D DMA salt	2618	8/7/08	8/11/08
R050804-14	Mature Fodder	2,4-D DMA salt	7123	9/3/08	9/8/08
R050804-15	Mature cobs	2,4-D DMA salt	1276	9/3/08	9/8/08
R050804-16	Mature grain	2,4-D DMA salt	1347	9/3/08	9/8/08

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-2,4-D DMA salt (150 mL)	1	1	26309.71	26068.67	92.61
		2	26041.67		
		3	26103.13		
		4	26128.04		
		5	26397.93		
		6	26197.09		
		7	25796.60		
		8	25844.24		
		9	25799.65		
^{14}C-2,4-D DMA salt (150 mL)	2	1	26333.26	26493.29	94.12
		2	26051.58		
		3	26326.81		
		4	26364.58		
		5	27371.67		
		6	26068.33		
		7	26695.72		
		8	26464.90		
		9	26762.74		
^{14}C-2,4-D DMA salt (200 mL)	3	1	23057.57	22654.02	107.31
		2	22786.79		
		3	22695.22		
		4	22646.06		
		5	22437.00		
		6	22447.57		
		7	22618.97		
		8	22596.49		
		9	22600.51		

[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 2,4D-DMA salt
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. 63897
Dow Study No. 080058

ABC Study No. 63897
DAS Study No. 080058

STATEMENT OF GLP COMPLIANCE

Compound: 2,4D-DMA salt

Study Title: A Nature of the Residue Study with 2,4D-DMA salt 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 29 Dec 08
Jon E. Rhodes, MS Date
Director
Chemical Services
ABC Laboratories, Inc.

Sponsor:

03/May/2010
Sandra Rotondaro Date
Study Director
Dow AgroSciences, LLC

Submitter:

January 12, 2010
D. Fonseca Date
Regulatory Manager
Dow AgroSciences, LLC

ABC Study No. 63897
DAS Study No. 080058

QUALITY ASSURANCE STATEMENT

ABC's Quality Assurance Unit reviewed the Study No. 63897 entitled "A Nature of the Residue Study with 2,4D-DMA salt 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
22 Sep 08	Procedure: Sample Preparation	23 Sep 08	24 Sep 08	01 Oct 08
29 Sep 08	Procedure: Sample Combustion	06 Oct 08	22 Oct 08	28 Oct 08
02 Dec 08	Raw Data & Draft Sample Processing Report	02 Dec 08	17 Dec 08	17 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 27 April 2010

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

Date

ABC Study No. 63897
DAS Study No. 080058

SIGNATURE PAGE

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

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

29 Dec 08
Date

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

29 Dec 08
Date

ABC Study No. 63897
DAS Study No. 080058

SAMPLE PROCESSING SUMMARY REPORT

Study Sponsor:	Dow AgroSciences, LLC
Study Title:	A Nature of the Residue Study with 2,4D-DMA salt 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, cobs, fodder, 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 TRR analysis. Weighing and milling procedures were documented using ABC Laboratories' "Daily Sample Preparation Log" form.

All samples were broken down using the Robot Coupe cutter/mixer and milling completed on the Straub grinding mill with the exception of Sample No. R050804-13 which was milled using only the Robot Coupe cutter/mixer. 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 containers(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 containers(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. Overall, combustion results indicated that treated samples were homogeneous with CVs of no greater than 10%.

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 Carbon-14 cocktail (R.J. Harvey Instruments Corporation, Tappan, NJ).

The homogenized maize 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.

ABC Study No. 63897
DAS Study No. 080058

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

Table 1 Sample Information for Corn Fractions from Plots Treated with 2,4D-DMA Salt

Sample ID	Matrix	Plot	Sampling Time Point	Date Received at ABC	Date Prepared	Date of TRR Determination	Date Shipped to DAS
R050804-13	Corn plant	2,4D-DMA Salt	Forage	12 Aug 08	19 Aug 08	21 Aug 08	02 Sep 08
R050804-14	Corn fodder	2,4D-DMA Salt	Mature	10 Sep 08	02 Oct 08	06 Oct 08	08 Oct 08
R050804-15	Corn cobs	2,4D-DMA Salt	Mature	10 Sep 08	22 Sep 08	30 Sep 08	01 Oct 08
R050804-16	Corn grain	2,4D-DMA Salt	Mature	10 Sep 08	22 Sep 08	30 Sep 08	01 Oct 08

Table 2 Combustion Results in Corn Fraction Samples for Corn Matrices from Plots Treated with 2,4D-DMA Salt

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
R050804-13	Treated Corn Forage	21 Aug 08	1	98.3	0.202	18833.07	50.80	18782.27	94636.25	106359.55
		21 Aug 08	2		0.203	22158.42		22107.62	110842.62	
		21 Aug 08	3		0.208	22062.64		22011.84	107709.45	
		21 Aug 08	4		0.208	21492.45		21441.65	104919.37	
		21 Aug 08	5		0.211	23619.96		23569.16	113690.05	
R050804-14	Treated Corn Fodder	06 Oct 08	1	100.0	0.200	33291.96	53.54	33238.42	166192.12	167579.08
		06 Oct 08	2		0.202	34063.61		34010.07	168366.70	
		06 Oct 08	3		0.203	34367.00		34313.46	169031.84	
		06 Oct 08	4		0.200	32484.36		32430.82	162154.12	
		06 Oct 08	5		0.204	35172.26		35118.72	172150.60	
R050804-15	Control Corn Cobs	30 Sep 08	1	97.2	0.203	178.52	32.58	145.94	739.61	777.34
		30 Sep 08	2		0.205	193.76		161.18	808.87	
		30 Sep 08	3		0.201	167.31		134.73	689.59	
		30 Sep 08	4		0.204	190.14		157.56	794.58	
		30 Sep 08	5		0.201	199.44		166.86	854.04	
R050804-16	Treated Corn Grain	30 Sep 08	1	97.2	0.208	277.97	32.58	245.39	1213.72	1219.07
		30 Sep 08	2		0.204	255.85		223.27	1125.96	
		30 Sep 08	3		0.200	240.98		208.40	1071.99	
		30 Sep 08	4		0.204	297.05		264.47	1333.74	
		30 Sep 08	5		0.201	296.33		263.75	1349.96	

[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 2,4-D DMA in the test substance. First, the total amount of radioactive 2,4-D DAM was determined (dpm and µg). Then the specific activity was calculated as the sum of the radioactivity divided by the sum of the mass.

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

$$\text{Total Radioactive 2,4-D DMA}_{(\mu\text{g})} = \frac{\text{Total Radioactivity}_{\text{dpm}}}{\text{Original Specific Activity}_{\text{dpm/µg}}}$$

$$\text{New Specific Activity}_{(\text{dpm/µg})} = \frac{\text{total radioactivity}_{\text{dpm}}}{\text{total mass 2,4 - D DMA (radioactive + non - radioactive)}_{\mu\text{g}}}$$

Example for ^{14}C-2,4-D DMA:
Where the radiolabeled test substance (nominally 5.8 mCi) was diluted to 10.0 mL and 0.025 mL aliquots were diluted to 20.0 mL, and 0.025 mL aliquots were taken for LSC. The average dpm/aliquot was 41,137 dpm/0.025 mL. The original specific activity was 362,047 dpm/µg. The majority of the test substance solution, 9.925 mL, was mixed with 491.1 mg non-radiolabeled 2,4-D (99.0% purity resulting in 486.2 mg or 486,189 µg).

$$\text{Total Radioactivity}_{(\text{dpm})} = \left(\frac{41137\ \text{dpm}}{0.025\ \text{mL}}\right) \times \left(\frac{20\ \text{mL}}{0.025\ \text{mL}}\right) \times 9.925\ \text{mL} = 1.31 \text{ x } 10^{10}\ \text{dpm}$$

$$\text{Total Radioactive 2,4-D} = \frac{1.31^{10}\ \text{dpm}}{362047\ \text{dpm/µg}} = 36{,}087\ \mu\text{g (99.711\% pure = 35,983 µg)}$$

$$\text{Specific Activity 2,4-D}_{(\text{dpm/µg})} = \frac{1.31 \times 10^{10}\ \text{dpm}}{35{,}983\ \mu\text{g} + 486{,}189\ \mu\text{g}} = 25{,}021\ \text{dpm/µg}$$

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 forage (replicate A) post-extracted pellet combustion:
Where oxidizer recovery = 97.7%, combustion value #1 = 6168 dpm, and aliquot weight = 0.0998 g.

$$\text{Net dpm value} = \frac{6168\ \text{dpm}}{0.977 \times 0.0998\ \text{g}} = 63{,}259\ \text{dpm/g}$$ (rounding difference noted)

Calculation of TRR Levels

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

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

To determine the total radioactive residue level in each sample, the average dpm/g value for the sample was converted to µg active ingredient/g (equivalent to mg a.i./kg) by dividing the dpm/g value by the specific activity value of the applied ^{14}C-2,4-D DMA (25,021 dpm/µg).

For example, average dpm/g in the forage was 106,360. This was converted to a µg ai/g (or mg ai/kg) value as follows:

$$\frac{106{,}360\ \text{dpm/g}}{25{,}021\ \text{dpm/µg}} = 4.251\ \text{µg a.i./g (or mg a.i./kg)}$$

c) *Converting mg ai/kg to mg ae/kg*

To determine the total radioactive residue level in each sample in terms of acid equivalents, the mg ai/kg value was multiplied by a conversion factor. The conversion factor was calculated by dividing the molecular weight of the acid by the molecular weight of the active ingredient (2,4-D DMA), in this case, 221.04/266.12 = 0.831 (see

Figure 2).

For example, forage contained 4.251 mg a.i./kg) and was converted to mg a.e./kg as follows:

$$4.251\,\text{mg a.i./kg} \times 0.831 = 3.531\ \mu\text{g a.e./g (or mg a.e./kg) (rounding difference noted)}$$

d) *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 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 forage (replicate A):

$$\text{Amount Extracted (dpm)} = \frac{1812\ \text{dpm}}{0.25\ \text{mL}} \times 136\ \text{mL} = 985{,}909\ \text{dpm}$$ (rounding difference noted)

$$\text{Extraction Recovery} = \frac{985{,}909\ \text{dpm}}{10.0\ \text{g x}\ 106{,}360\ \text{dpm/g}} = 92.7\%$$ (rounding difference noted)

Extracted mg ae/kg = 0.927 x 3.531 mg a.e./kg = 3.273 mg/kg

$$\text{Normalized Extraction Recovery} = \frac{3.273\ \text{mg/kg}}{3.273\ \text{mg/kg} + 0.289\ \text{mg/kg} + 0.315\ \text{mg/kg}} = 84.4\%$$

(where 0.289 and 0.315 mg a.e./kg are the amounts in EX2 and the post-extracted pellet, respectively)

e) *TRR Distribution among 2,4-D and Its Metabolites Following HPLC Analysis*

The percentage distribution of the TRR among 2,4-D 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 9, the percent of the TRR accounted for as parent 2,4-D in the Neutral Organic extract of the forage (replicate A):

% of TRR = 92.7% x 0.703 (70.3% of the radioactivity in this extract that eluted with 2,4-D – see Figure 8)
= 65.1% of the TRR (rounding difference noted)

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 2,4-D acid equivalents) was multiplied by the percentage value at which the component of interest was present.

For the 2,4-D in the above sample the calculation:
2,4-D = 65.1% of the TRR x 3.531 mg a.e./kg (TRR – see Table 7)
= 2.300 mg a.e./kg

Appendix D—Mass Spectral Report

Mass Spectral Analysis Summary

Two radiolabeled samples and two reference standards from *A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008* (Protocol ID: 080058) were submitted for mass spectral analysis. Two compounds of interest were detected in each of the analyzed samples and are discussed in this report.

Liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) were used to analyze the samples and the standards. A Berthold radioactivity monitor (RAM) was used to assist in location of the metabolite peaks.

Calibration standard

Prior to analysis, calibration of the Thermo LTQ FT Ultra mass spectrometer was verified using a solution of caffeine, the tetrapeptide MRFA, and Ultramark 1621.

Mass Measurement Reporting

Mass measurements made using the ion trap (IT-MS) for the MS and MS/MS mass spectra were measured to +/- 0.1 Da accuracy (for example *m/z* 300.1 +/- 0.1). Mass measurements made using the FTICR (FT-MS) for the MS and MS/MS mass spectra were measured to +/- 0.001 Da accuracy (for example, *m/z* 300.001 +/- 0.001). The reporting of these values is based on the typical known accuracy limitations of each mass analyzer.

Chromatographic and Mass Spectral Parameters

The LC, MS, and MS/MS parameters used to analyze the samples and reference standards from *A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008* (Protocol ID: 080058) are shown in **Table 1**. The mass spectrometer instrumental parameters including gas flow and applied voltages were optimized prior to the analyses.

Table 1. Chromatographic and Mass Spectral Parameters

Instrumentation	Mass Spectrometer	Thermo LTQ FT Ultra, s/n: SN06120F	
	HPLC	Thermo Accela	
Liquid Chromatography	Column	Supelco Ascentis C_{18}, 150 x 4.6 mm, 2.7 micron, serial number: USHW002792	
	Solvent A	0.1% formic acid in water	
	Solvent B	0.1% formic acid in acetonitrile	
	Flow rate	1.0 mL/minute	
	HPLC split flow ratio	Approximately 20:80 (MS:RAM)	
LC Gradient	Time (minutes)	% Solvent A	% Solvent B
	0.00	95	5
	5.00	95	5
	20.00	50	50
	25.00	50	50
	40.00	5	95
	42.00	5	95
	43.00	95	5
	50.00	95	5
MS and MS/MS parameters	Mode	Negative electrospray (-ESI)	
	MS/MS Isolation width	5.0 Da	
	CE	35%	
	Activation Q	0.250	
	Activation Time	30.0 msec	

Mass Spectral Results and Discussion

A summary of the mass spectral results for the reference standards that were analyzed as part of this work is shown in **Table 2**. The mass spectral results for the sample analyses are shown in **Tables 3** and **4.**

The RAM trace depicting **peak A** and **peak C** in sample *FOR-A+B-SiOH5-8* is shown in **Figure 1.** The RAM trace depicting **peak B** and **peak C** in sample *FOR-A+B-SiOH4* is shown in **Figure 2.**

Peak A in the sample *FOR-A+B-SiOH5-8*, which eluted at approximately 13.20 minutes (RAM trace), produced a deprotonated formate adduct ion, $[M-H+formate]^-$, at *m/z*: 531.067 +/- 0.001 under negative ESI conditions. The chromatographic and mass spectral results for **peak A** are shown in **Figure 3**. This accurate mass measurement for the unknown (not adduct) was 486.069 Da which was consistent with the molecular formula $C_{18}H_{24}Cl_2O_{11}$. This formula is consistent with a structure containing 6 rings and double-bonds. Based on this evidence, the structure presented in **Table 3** has been proposed for **peak A**. The proposed structure for **Peak A** is a disaccharide conjugate of dichlorophenol (DCP) metabolite of 2,4-D. A reference standard of this compound was not available for chromatographic and mass spectral comparison.

Peak C in the sample *FOR-A+B-SiOH5-8*, which eluted at approximately 20.55 minutes (RAM trace), was compared to the standard of 2,4-D (AGR275828). The chromatographic and mass spectral results for both **peak C** and 2,4-D are shown in **Figure 4. Peak C** was identified as 2,4-D parent based upon relative retention time and mass spectral match with the known standard.

Peak B in the sample *FOR-A+B-SiOH4*, which eluted at approximately 15.03 minutes (RAM trace), was compared to the standard of X11963417 (DE3-100044-30). The chromatographic and mass spectral results for both **peak B** and X11963417 are shown in **Figure 5. Peak B** was identified as X11963417, the glucose conjugate of DCP based upon relative retention time and mass spectral match with the known standard.

Peak C in the sample *FOR-A+B-SiOH4*, which eluted at approximately 20.59 minutes (RAM trace), was compared to the standard of 2,4-D (AGR275828). The chromatographic and mass spectral results for both **peak C** and 2,4-D are shown in **Figure 6. Peak C** was identified as 2,4-D parent based upon relative retention time and mass spectral match with the known standard.

Table 2. Summary of mass spectral results observed from the reference standards used in *A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008*

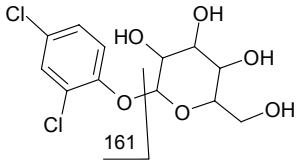
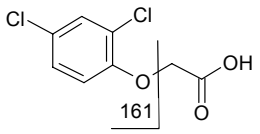
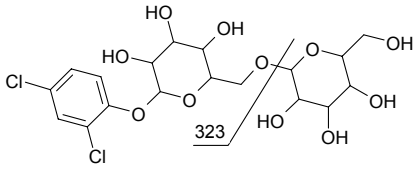
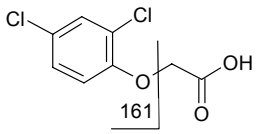
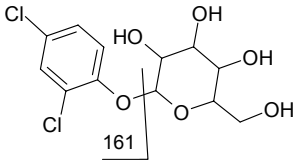
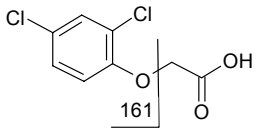
Standard	**-ESI/MS *m/z*** **-ESI/MS/MS *m/z***	**Structure** **MW (Da) and Formula**
DE3-100044-30 RT: 15.00 min (MS) *X11963417* *(glucose conjugate of dichlorophenol, metabolite of 2,4-D)*	369.015 $[M-H+formate]^-$ 160.9, 322.9	 324, $C_{12}H_{14}Cl_2O_6$
AGR275828 RT: 20.54 min (MS) *2,4-D*	264.967 $[M-H+formate]^-$ 161.0	 220, $C_8H_6Cl_2O_3$

Table 3. Summary of mass spectral results observed from *A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008* sample *FOR-A+B-SiOH5-8*

Peak ID **Retention Time (min)** **RAM / MS**	**-ESI/MS *m/z*** **-ESI/MS/MS *m/z***	**Proposed Structure [1,2]** **MW (Da) and Formula**
A 13.20 / 13.24	531.067 $[M-H+formate]^-$ 323.2, 484.9	 Or isomer 486, $C_{18}H_{24}Cl_2O_{11}$
C 20.55 / 20.53	264.967 $[M-H+formate]^-$ 160.9	 220, $C_8H_6Cl_2O_3$

[1] Peak A: structure assignment based on mass assignment and MS/MS fragments.
[2] Peak B: structure assignment based upon relative LC retention time and mass spectral match to a reference standard.

Table 4. Summary of mass spectral results observed from *A Nature of the Residue Study with [14C]-2,4-D Applied to AAD-1 Corn, 2008* sample *FOR-A+B-SiOH4*

Peak ID **Retention Time (min)** **RAM / MS**	**-ESI/MS *m/z*** **-ESI/MS/MS *m/z***	**Proposed Structure [1]** **MW (Da) and Formula**
B 15.03 / 14.99	369.015 $[M-H+formate]^-$ 161.0, 323.0	 324, $C_{12}H_{14}Cl_2O_6$
C 20.59 / 20.54	264.967 $[M-H+formate]^-$ 161.0	 220, $C_8H_6Cl_2O_3$

[1]Structure assignment based upon relative LC retention time and mass spectral match to a reference standard.

Figure 1. Chromatographic results showing the RAM trace for the sample *FOR-A+B-SiOH5-8*

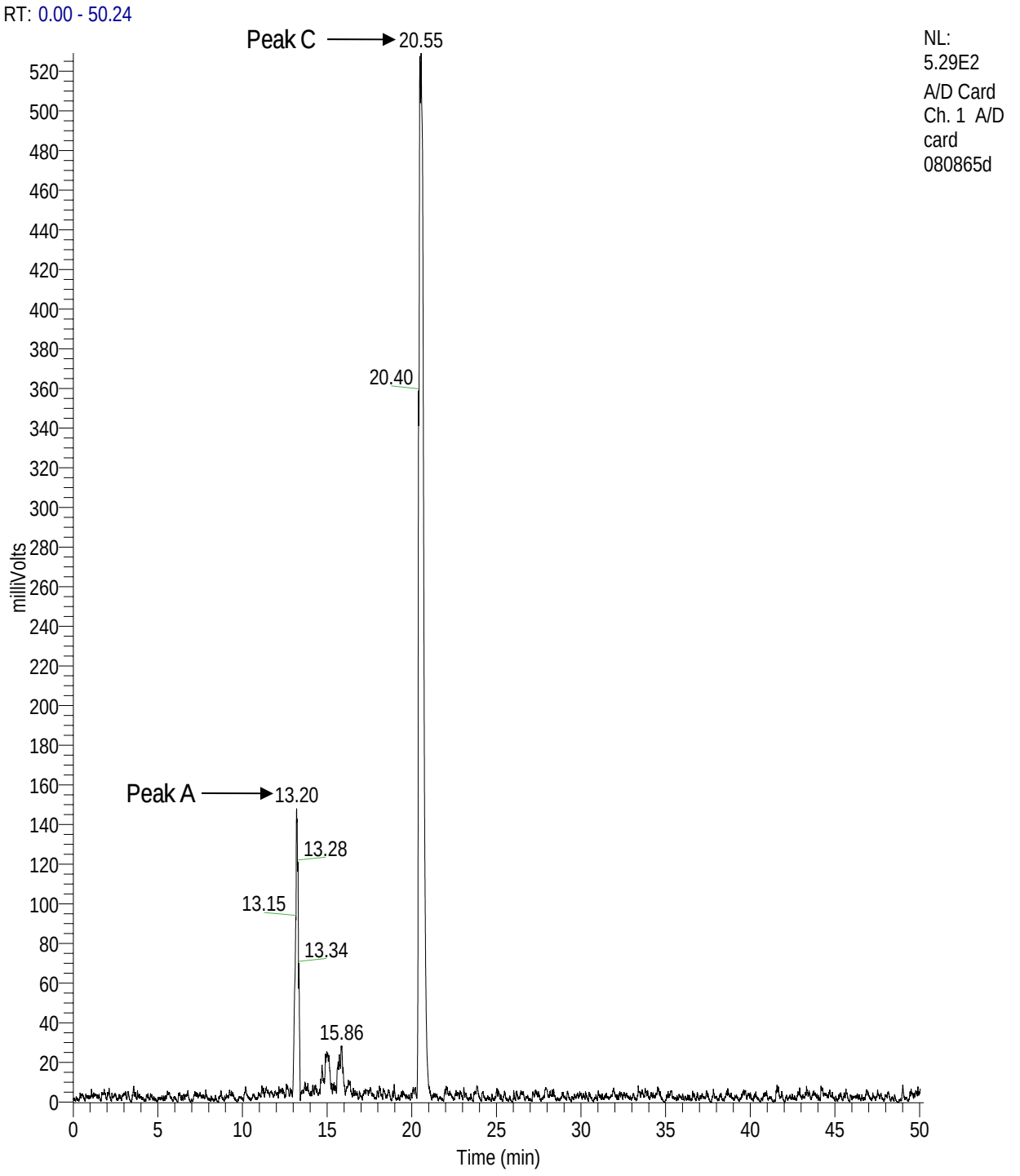


Figure 2. Chromatographic results showing the RAM trace for the sample *FOR-A+B-SiOH4*

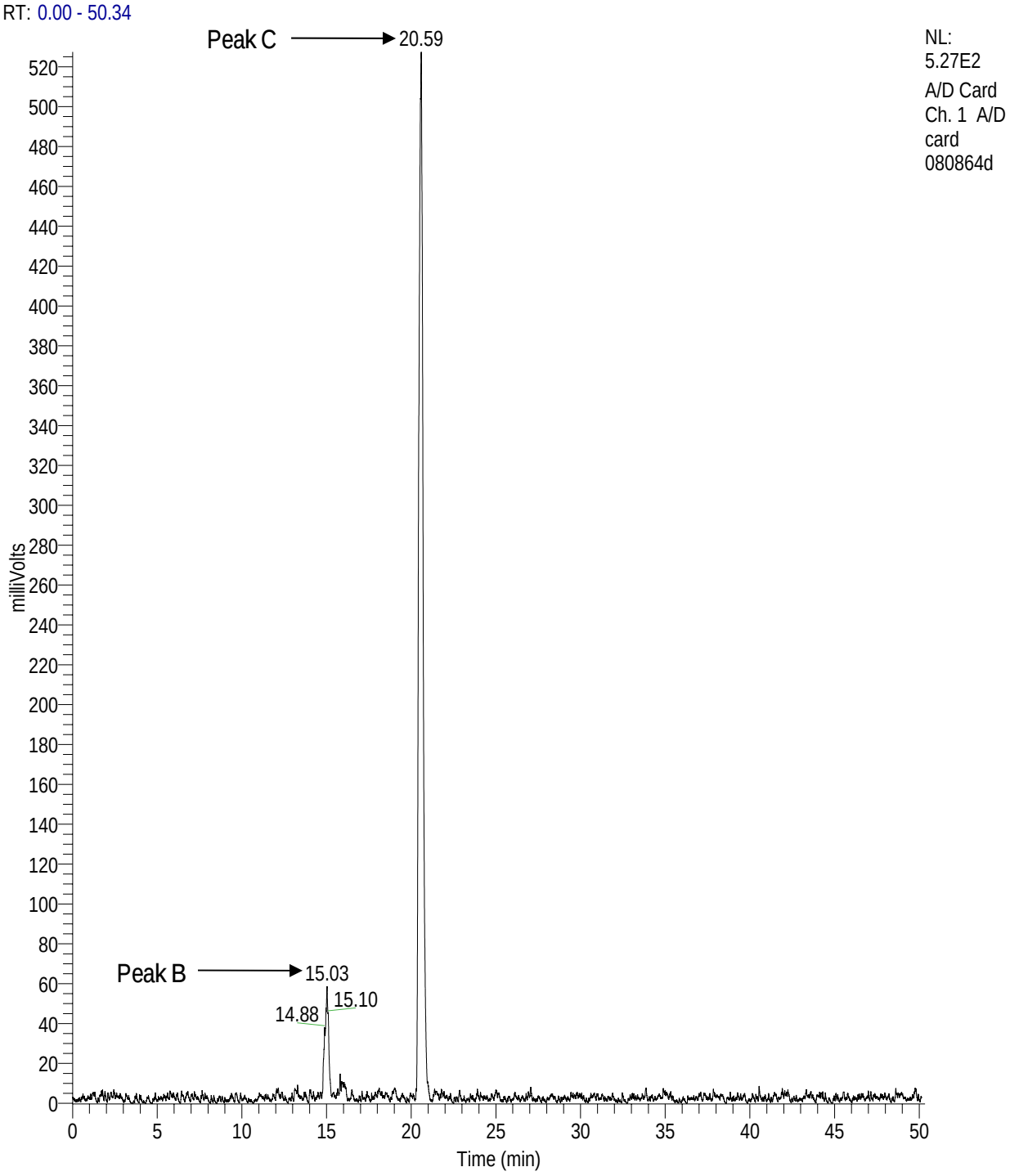
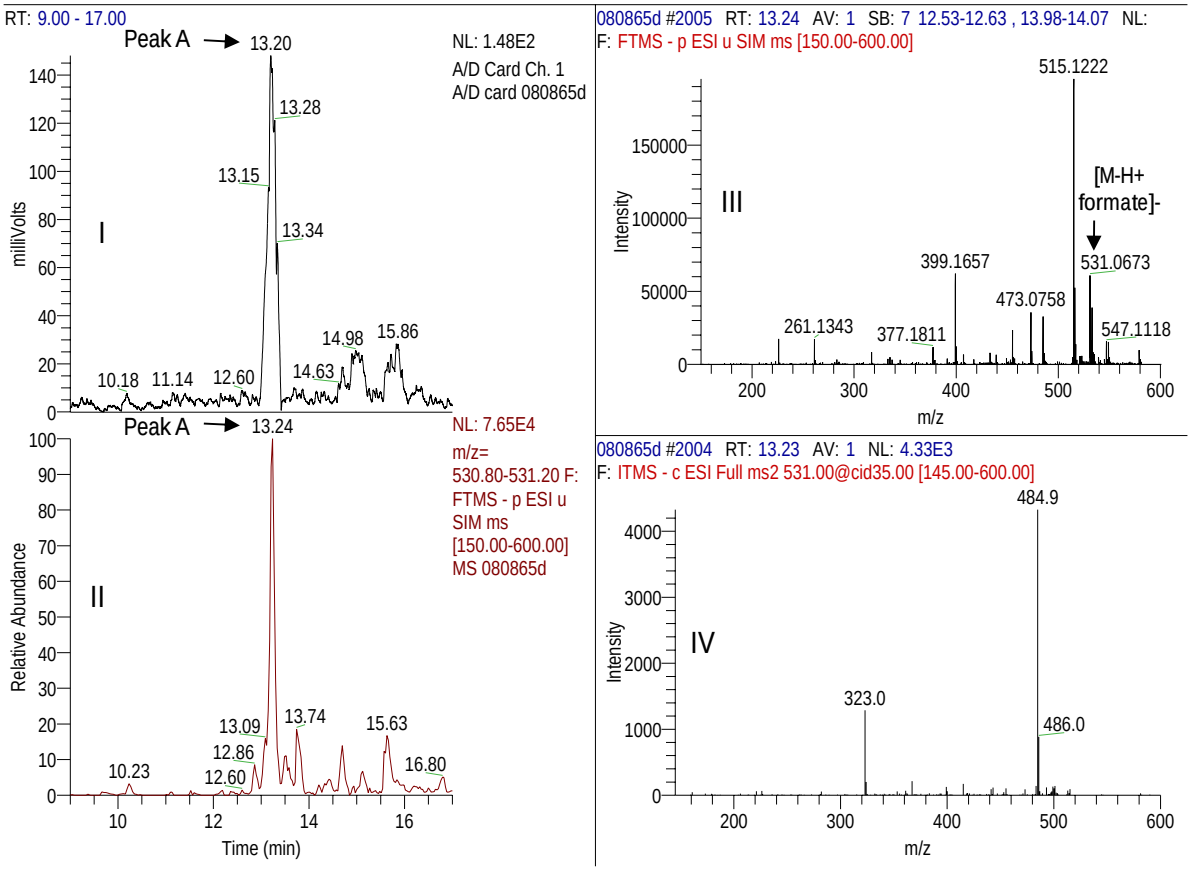


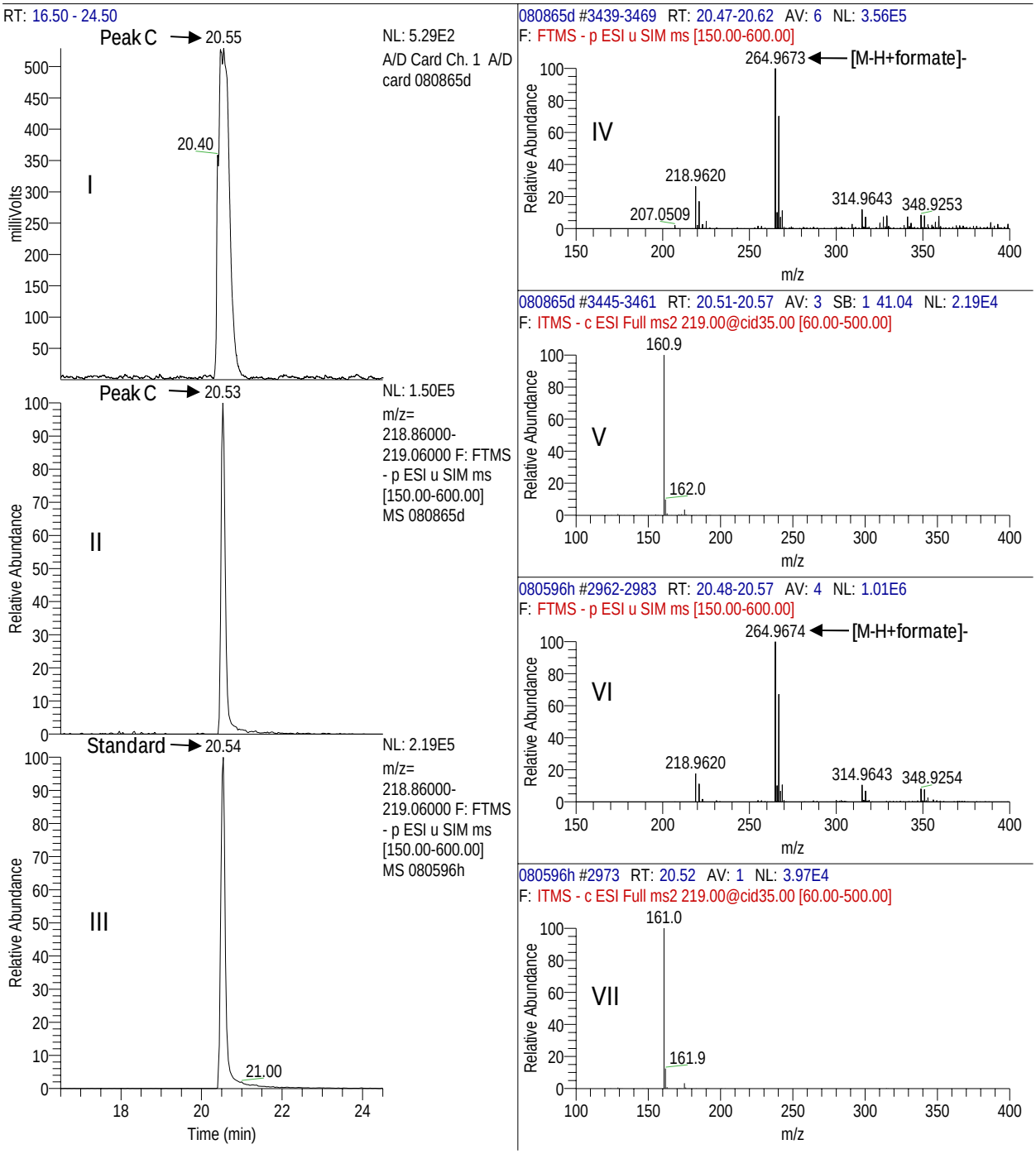
Figure 3. Chromatographic and mass spectral results for **peak A** in sample *FOR-A+B-SiOH5-8*



Observed $[M-H+formate]^-$	531.067
Observed M (-formate+H: 44.998)	486.069
Proposed Theoretical M	486.070
Measured Error from Proposed Theoretical M	0.001 Da or 2 ppm
Proposed Molecular Formula	$C_{18}H_{24}Cl_2O_{11}$
Rings and Double Bonds for Proposed Formula	6

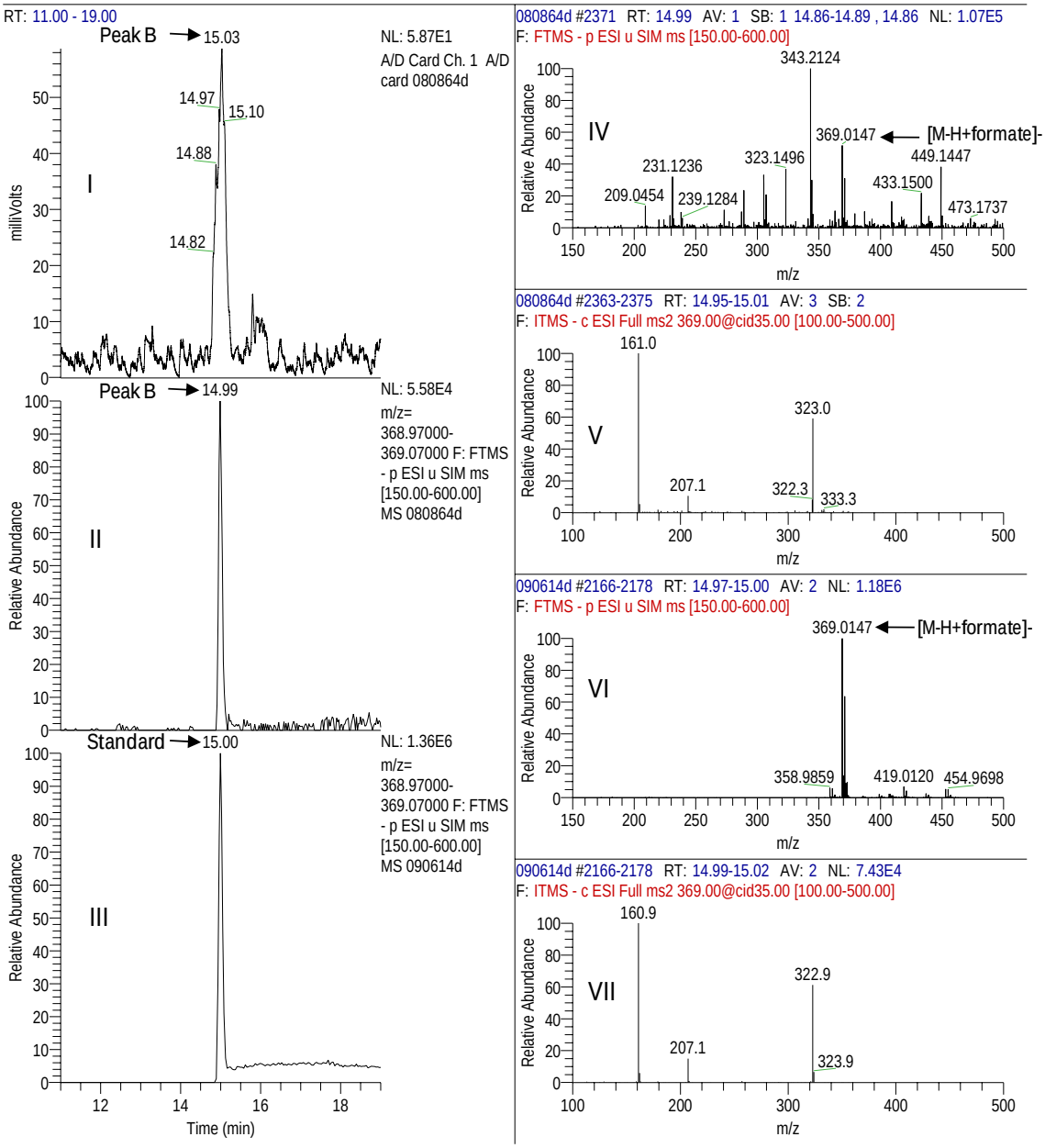
I-II: sample – RAM, extracted ion chromatogram
III-IV: sample – mass spectrum, MS/MS spectrum

Figure 4. Chromatographic and mass spectral results for **peak C** in sample *FOR-A+B-SiOH5-8* and 2,4-D standard (*AGR275828)*



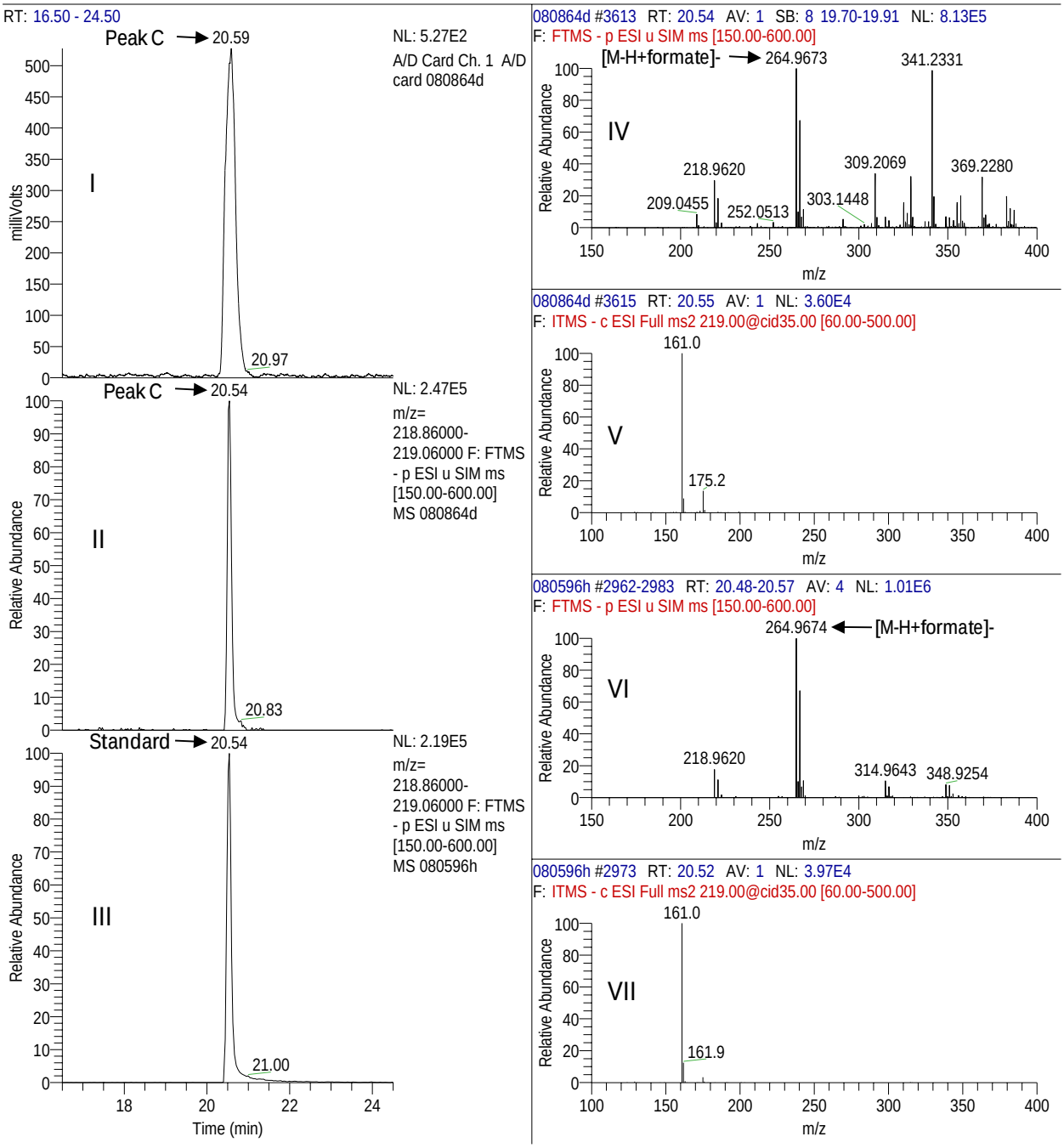
I-II: sample – RAM, extracted ion chromatogram
III: standard – extracted ion chromatogram
IV-V: sample – mass spectrum, MS/MS spectrum
VI-VII: standard – mass spectrum, MS/MS spectrum

Figure 5. Chromatographic and mass spectral results for **peak B** in sample *FOR-A+B-SiOH4* and standard of X11963417 (DE3-100044-30)



I-II: sample – RAM, extracted ion chromatogram
III: standard – extracted ion chromatogram
IV-V: sample – mass spectrum, MS/MS spectrum
VI-VII: standard – mass spectrum, MS/MS spectrum

Figure 6. Chromatographic and mass spectral results for **peak C** in sample *FOR-A+B-SiOH4* and 2,4-D standard (*AGR275828)*



I-II: sample – RAM, extracted ion chromatogram
III: standard – extracted ion chromatogram
IV-V: sample – mass spectrum, MS/MS spectrum
VI-VII: standard – mass spectrum, MS/MS spectrum