Study Title

Amended Report for MSL 0022681: Assessment of CP4 EPSPS Protein Levels in Canola Tissues Collected from MON 88302 Produced in United States and Canadian Field Trials during 2009

Authors

[redacted]

Study Completed On

April 22, 2010

Amended Report Completed On

November 19, 2010

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Laboratory Project ID

Monsanto Study Number: REG-10-107
Report Number: MSL0023090

The text below applies only to use of the data by the United States Environmental Protection Agency (U.S. EPA) in connection with the provisions of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

The inclusion of this page in all studies is for quality assurance purposes and does not necessarily indicate that this study has been submitted to the U.S. EPA.

Statement of No Data Confidentiality Claim

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

We submit this material to the U.S. EPA specifically under the requirements set forth in FIFRA as amended, and consent to the use and disclosure of this material by the EPA strictly in accordance with FIFRA. By submitting this material to the EPA in accordance with the method and format requirements contained in PR Notice 86-5, we reserve and do not waive any rights involving this material that are or can be claimed by the company notwithstanding this submission to the EPA.

Company: Monsanto Company

Company Agent

Title

Signature Date

Statement of Compliance

This study meets the U.S. EPA Good Laboratory Practice requirements as specified in 40 CFR Part 160 with the following exception: Stability of CP4 EPSPS in canola tissues has not been assessed.

Submitter Date

[redacted]

Study Director Date

Quality Assurance Statement

Study Title: Assessment of CP4 EPSPS Protein Levels in Canola Tissues Collected from MON 88302 Produced in United States and Canadian Field Trials during 2009

Study Number: REG-10-107

Reviews conducted by the Quality Assurance Unit confirm that the final report accurately describes the methods and standard operating procedures followed and accurately reflects the raw data of the study.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the study reported herein.

Dates of Inspection / Audit	Phase	Date Reported To: Study Director	Management
04/06/2010	Moisture Analysis	04/09/2010	04/09/2010
04/19/2010	Draft Report and Data Audit	04/21/2010	04/21/2010
10/25/2010	Report Amendment Audit	10/26/2010	10/26/2010

[redacted]

Monsanto Regulatory, Monsanto Company

Study Certification

This report is an accurate and complete representation of the study/project activities.

Signatures of Approval:

[redacted]

Study Director

[redacted]

Regulatory Product Characterization Center

Study Information

Study Number:	REG-10-107
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Title:	Amended Report for MSL 0022681: Assessment of CP4 EPSPS Protein Levels in Canola Tissues Collected from MON 88302 Produced in United States and Canadian Field Trials during 2009
Facility:	Monsanto Company Regulatory Product Characterization Center 800 North Lindbergh Boulevard Saint Louis, Missouri 63167
Study Director:	[redacted]
Team Lead:	[redacted] Protein Expression Platform
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Study Initiation Date:	March 19, 2010
Study Completion Date:	April 22, 2010
Amended Final Report Completion Date:	November 19, 2010
Records Retention:	*The protocol, all raw data (including rejected data), documentation, records, and the final report for this study is retained at Monsanto Company.*

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Abbreviations[1] and Definitions

a.e.	acid equivalent
BBCH	Bayer, BASF, Ciba-Geigy and Hoechst ceral grain growth scale
COC	chain-of-custody
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase derived from *Agrobacterium* sp. strain CP4
CV	coefficient of variation
DWCF	dry-weight conversion factor
Dwt	dry-weight basis
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
Fwt	fresh-weight basis
HRP	horseradish peroxidase
IgG	Immunoglobulin G
LOD	limit of detection
LOQ	limit of quantitation
n	number of samples
OSL	over-season leaf
PBST	phosphate-buffered saline containing Tween-20
PCR	polymerase chain reaction
RR	Roundup Ready®
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOP	standard operating procedure
TBA	Tris-Borate with L-Ascorbic Acid
TMB	3, 3′, 5, 5′- tetramethylbenzidine
Tris	tris(hydroxymethyl)aminomethane
TSSP	tissue-specific site pool

[1] Standard abbreviations, e.g., units of measure, were used in this report according to format described in "Instructions to Authors" in the Journal of Biological Chemistry.

1.0 Summary

Monsanto Company has developed a second generation herbicide-tolerant canola product, MON 88302 that allows a glyphosate application from emergence to first flowering at a rate up to 1.8 kg acid equivalent (a.e.) per hectare. With an increased window of application and higher spray rates, MON 88302 will provide superior weed control compared to the commercial first generation Roundup Ready® (RR) canola product RT73 (also referred to as GT73). MON 88302 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*). Expression of the CP4 EPSPS protein renders the plant tolerant to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

This study determined the expression levels of CP4 EPSPS protein by a validated enzyme-linked immunosorbent assay (ELISA) in tissues collected from MON 88302 produced in United States and Canadian field trials during 2009. Tissue samples were collected from plants grown in the United States at three field sites in 2009 under Production Plan PPN-08-462 and from plants grown in Canada at three field sites in 2009 under Production Plan PPN-08-586. In this study expression in forage, grain, over-season leaf (OSL1-4), and root (Root-1 and Root-2) tissues were determined by ELISA analysis. All protein levels for each tissue type were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was then measured for all tissue types and all protein levels were converted and reported on a dry weight (dwt) basis.

The mean CP4 EPSPS protein levels in MON 88302 across all sites were 170 µg/g dwt in forage and 27 µg/g dwt in grain. In tissues harvested throughout the growing season, mean CP4 EPSPS protein levels in MON 88302 across all sites ranged from 180 - 230 µg/g dwt in leaf, and 38 - 82 µg/g dwt in root.

2.0 Introduction

2.1 Background

Monsanto Company has developed a second generation herbicide-tolerant canola product, MON 88302 that allows a glyphosate application from emergence to first flowering at a rate up to 1.8 kg a.e. per hectare. With an increased window of application and higher spray rates, MON 88302 will provide superior weed control compared to the commercial first generation Roundup Ready® (RR) canola product RT73 (also referred to as GT73). MON 88302 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium sp*. strain CP4 (*cp4 epsps*). Expression of the CP4 EPSPS protein renders the plant tolerant to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

® Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.

CP4 EPSPS protein levels were determined in canola tissues produced at three United States field sites and three Canadian field sites in 2009. Canola was planted in a four replicate, randomized, complete block design at each field site.

2.2 Purpose

The purpose of this study was to determine the level of CP4 EPSPS protein in canola tissues collected from MON 88302 grown in the United States and Canada during 2009.

3.0 Materials

3.1 Test and Reference Substances

3.1.1 Test Substances

The test substance for this study was MON 88302. Tissue samples were collected as outlined in Production Plan PPN-08-462 and PPN-08-586 from plants grown from starting seed lot 11225246.

3.1.2 Characterization of Test Substances

The identities of the test substances were confirmed by verifying the chain-of-custody (COC) documentation prior to analysis. To further confirm the identities of the test substances, event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested grain from each site. The PCR analyses were archived in the Monsanto Regulatory Archives under the starting seed lot number described in section 3.1.1. Four grain samples tested unexpectedly during PCR verification. The four grain samples and their associated tissues were not used in this study.

3.1.3 Reference Substances

An *Escherichia coli* (*E. coli*) produced protein standard was used in this study and a copy of the certificate of analysis was archived with the study data. This CP4 EPSPS protein standard (lot 10000739) was used as the reference substance for analysis of CP4 EPSPS protein levels. The purity was 97% as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis. The purity-corrected protein concentration of the standard was 3.7 mg/ml as determined by amino acid composition analysis.

4.0 Methods

4.1 Generation of Plant Samples

4.1.1 Summary of Field Design

Production Plans PPN-08-462 and PPN-08-586 were initiated during the 2009 planting season to generate test substances from various canola growing locations in the United States and Canada, respectively. The tissue samples from the following United States field sites were used in this study: Power County, Idaho (IDAF); Wilkin County, Minnesota (MNCA); and McHenry County, North

Dakota (NDVA). The tissue samples from the following Canadian field sites were used in this study: Portage la Prairie, Manitoba (MBPL); Newton, Manitoba (MBNW); and Saskatoon, Saskatchewan (SKSA). These field sites were representative of canola producing regions suitable for commercial production. At each site, four replicated plots of plants containing MON 88302 were planted using a randomized complete block field design. Tissues were collected from each replicated plot at each field site. Tissue samples, except grain, from PPN-08-462 were stored and shipped at -80 °C. Grain samples were stored and shipped at ambient temperature.

Tissue samples, except grain and OSL-1 from MBPL site, from PPN-08-586 were stored and shipped at -20 °C. OSL-1 samples from the MBPL site were shipped and stored at -80 °C. The shipment and storage of samples at -20 °C was a deviation from the production plan, however there is no apparent impact from storing the tissues at -20 °C.

4.2 Tissue Processing and Protein Extraction Methods

4.2.1 Processing Method

All tissue samples produced at the field sites were shipped to Monsanto, St. Louis and were prepared by the Monsanto Sample Management Team. The prepared tissue samples were stored in a -80 °C freezer until transferred on dry ice to the analytical facility.

4.2.2 Extraction Methods

The CP4 EPSPS protein was extracted from canola tissues as described in Monsanto Standard Operating Procedure (SOP) AG-ME-1362-01 draft dated March 22, 2010. A copy of the draft SOP was archived with the study. Extraction parameters for the CP4 EPSPS protein and tissue types are described in Appendix 2. The extracts were aliquoted and stored in a -80 °C freezer until analysis.

4.3 ELISA Reagents and Methods

4.3.1 CP4 EPSPS Antibodies.

Mouse monoclonal antibody clone 39B6.1 (IgG2a isotype, kappa light chain; Orion lot 10002190) specific for the CP4 EPSPS protein was produced by Strategic Biosolutions (Newark, DE) and further purified from the mouse ascites fluid using Protein-A Sepharose affinity chromatography. The concentration of the purified IgG was determined to be 2.3 mg/ml by spectrophotometric methods. The purified antibody was stored in a buffer (pH 7.2) containing 0.02 M NaH_2PO_4, 0.15 M NaCl, and 15 ppm Proclin 300.

The detection reagent used was a goat anti-CP4 EPSPS antibody, otherwise known as anti-protein 4 (Sigma-Aldrich, catalog number P-5867) conjugated to horseradish peroxidase (HRP).

4.3.2 CP4 EPSPS ELISA Method

The CP4 EPSPS ELISA was performed manually according to SOP AG-ME-1362-01 draft dated March 22, 2010. Mouse anti-CP4 EPSPS antibody was diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) to a final concentration of 2.0 µg/ml, and immobilized onto 96-well microtiter plates followed by incubation in a 4 °C refrigerator for ≥8 h. Prior to each step in the assay, plates were washed with 1× phosphate-buffered saline containing 0.05% Tween-20 (PBST). CP4 EPSPS protein standard or sample extract was added at 100 µl per well and incubated for 1 h at 37 °C. The captured CP4 EPSPS protein was detected by the addition of 100 µl per well of anti-CP4 EPSPS HRP conjugate. Plates were developed by adding 100 µl per well of TMB. The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantification of the CP4 EPSPS protein was accomplished by interpolation on a CP4 EPSPS protein standard curve that ranged from 0.456-14.6 ng/ml.

4.3.3 CP4 EPSPS ELISA Validation

Appendix 2 summarizes the results of the validation of the ELISA used to determine the CP4 EPSPS protein levels in canola tissues.

4.4 Control of Bias

The test substances were planted using a randomized complete block design as described in Production Plans PPN-08-462 and PPN-08-586. The substances were randomly assigned to the plots within a block to prevent any experimental bias. Representative tissues from each plot were collected as described in the production plans. All tissues were processed by thoroughly grinding to produce a homogeneous sample before extraction to minimize sampling bias. The ELISA method used was optimized to minimize method bias. Protein extracts from the test substance were analyzed by ELISA with the appropriate protein standard and inter-assay negative and positive controls.

4.5 Moisture Analysis

Tissues were analyzed for moisture content using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO) according to SOP BR-ME-1238-01. The drying parameters are documented and archived with the study. A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a given site. These pools were prepared for each tissue in this study. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$DWCF = 1 - [Mean\ \%\ TSSP\ Moisture / 100]$$

The DWCF was used to convert protein levels assessed on a µg/g fresh weight (fwt) basis into levels reported on a µg/g dry weight (dwt) basis using the following calculation:

$$\text{Protein Level in Dry Weight} = \left(\frac{\text{Protein Level Fresh Weight}}{\text{DWCF}}\right)$$

The protein levels (ng/ml) that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

4.6 Data Analyses

All CP4 EPSPS ELISA plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP version 5.0.1 software. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a "µg/g fwt" basis for data that were greater than or equal to the LOQ. For each protein, this conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values in "µg/g fwt" were also converted to "µg/g dwt" by applying the DWCF. Microsoft Excel 2007 (Version 12.0.6535.5002) SP2 MSO (12.0.6535.5002) Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS protein levels in canola tissues. The sample mean, standard deviations, and range were also calculated by Microsoft Excel 2007.

Any test substance extract that resulted in an unexpectedly negative result by ELISA analysis was re-extracted twice for the protein of interest and re-analyzed by ELISA to confirm the results. Samples with confirmed unexpected results were omitted from all calculations.

4.7 Protocol Amendments/Deviations

The protocol was amended to re-open the study in order to amend the final report. The amendment allowed for the removal of Grain and Root-2 tissues from the IDAF site. These tissues were removed from the analysis due to Production Plan PPN-08-462 Amendment 9 which removed the Grain and Root-2 tissues from the IDAF site because of an impact on the sample quality and quantity due to weather. All data associated with these tissues were rejected and therefore the expression summaries and final report were amended to reflect the changes in the data. This amendment resulted in a decreased sample number for Grain and Root-2 tissues for the sample mean, range and standard deviation. This amendment had a positive

impact on the study as the protein levels reported were the result of analysis of quality samples. The overall conclusions of the report were not changed.

5.0 Results

The across-site mean, standard deviation (SD), and range are reported for CP4 EPSPS protein levels on a µg/g fwt and µg/g dwt basis in canola tissues collected from three United States and three Canadian field sites in 2009 as described in Table 1.

5.1 Protein Levels in CP4 EPSPS

The CP4 EPSPS protein levels for MON 88302 are presented in Table 1. Results showed that the mean CP4 EPSPS protein levels in MON 88302 across all sites were 170 µg/g dwt in forage and 27 µg/g dwt in grain. In tissues harvested throughout the growing season, mean CP4 EPSPS protein levels in MON 88302 across all sites ranged from 180-230 µg/g dwt in leaf, and 38-82 µg/g dwt in root. One Root-2 sample from the MBNW site tested unexpectedly negative. The result was confirmed by re-extracting the sample twice and re-analyzing by ELISA. The sample was omitted from the calculations.

5.2 Stability of Test Materials

Tissue storage stability for CP4 EPSPS in processed canola tissues is being determined, and the data associated with the protein stability assessment will be archived under the current version of the Standard Operating Procedure (SOP) AG-ME-1362.

6.0 Conclusions

MON 88302 was grown in United States and Canada at three field sites from each country during the 2009 growing season. Tissue samples were collected and analyzed for CP4 EPSPS protein levels using a validated ELISA method. These data provide an estimation of the protein levels for CP4 EPSPS protein on a fresh weight and dry weight basis in eight tissue types throughout the growing season.

7.0 Acknowledgments

The authors would like to acknowledge Jack Milligan and the Agronomy and Sample Processing Center for processing the tissue samples and Andre Van Oyen, Jr., Tiffany Ernst, and John Lake from the Sample Dispensary for the sample distributions.

Table 1. Summary of CP4 EPSPS Protein Levels in Tissues Collected from MON 88302 Produced in United States and Canadian Field Trials in 2009

Tissue Type[1]	MON 88302	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]
Forage	18 (4.4) 14-28	170 (22) 120-210
Grain	25 (5.2) 21-43	27 (5.6) 22-46
OSL-1	23 (10) 10-45	180 (40) 110-250
OSL-2	22 (5.9) 18-37	180 (41) 120-250
OSL-3	31 (6.3) 20-41	230 (50) 130-300
OSL-4	36 (14) 20-85	210 (80) 110-500

Continued on next page

Table 1. Summary of CP4 EPSPS Protein Levels in Tissues Collected from MON 88302 Produced in United States and Canadian Field Trials in 2009 (continued)

Tissue Type[1]	MON 88302	
	Mean (SD)[2] Range[3] (µg/g fwt)[4]	Mean (SD) Range (µg/g dwt)[5]
Root-1	19 (4.1) 11-25	82 (17) 46-100
Root-2	10 (3.3) 7.0-17	38 (14) 24-62

1. Tissues were collected at the following growth stages (Bayer, BASF, Ciba-Geigy and Hoechst ceral grain growth scale):
   a. Forage 30 BBCH Growth Stage
   b. Grain 99 BBCH Growth Stage
   c. OSL-1 13-14 BBCH Growth Stage
   d. OSL-2 17-19 BBCH Growth Stage
   e. OSL-3 30 BBCH Growth Stage
   f. OSL-4 60-62 BBCH Growth Stage
   g. Root-1 30 BBCH Growth Stage
   h. Root-2 71-73 BBCH Growth Stage
2. The mean and standard deviation were calculated. The "n" values for the calculated mean and standard deviations represent the number of samples figured into the calculation.
   a. Forage n=20
   b. Grain n=16
   c. OSL-1 n=16
   d. OSL-2 n=9
   e. OSL-3 n=20
   f. OSL-4 n=20
   g. Root-1 n=19
   h. Root-2 n=11
   The following samples were not received into this study: OSL-1 tissue samples from site MBNW, OSL-2 tissue samples from sites MNCA, MBNW, and MBPL, the Root-1 tissue sample from site SKSA plot 104, Root-2 tissue samples from site SKSA, and the Root-2 tissue sample from site NDVA plot 209. One Root-2 sample from site MBNW tested unexpectedly by ELISA. Grain samples from site MNCA plot 110, NDVA plot 106, NDVA plot 302, and SKSA plot 207 tested unexpectedly during PCR verification. These tissue samples and all associated tissue samples were not analyzed in this study. Grain and Root-2 samples from site IDAF were excluded from the study.
3. Minimum and maximum values were determined for each tissue type.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt) basis. The dry weight values were calculated by dividing the µg/g fwt by the dry weight conversion factors obtained from moisture analysis data.

Appendices

Appendix 1. Standard Operating Procedures

AG-ME-1362-01	Extraction and Direct ELISA Analysis of CP4 EPSPS in Canola Tissues
BR-ME-1238-01	Analysis of Moisture Content Using the Denver Instrument IR-200 Moisture Analyzer

Appendix 2. Summary of the Validation Results for the CP4 EPSPS Protein ELISA in Canola Tissues

1.0 Accuracy

1.1 Extraction Efficiency and Spike and Recovery

Extraction Efficiency acceptance criteria = 70-130%.
Spike and Recovery acceptance criteria = 70-130%.

Tissue	Tissue-to-Buffer Ratio	Extraction Efficiency (%)[1]	Spike and Recovery (%)[2]
Leaf	1:100	87	104
Root	1:100	80	104
Forage	1:100	91	91
Grain	1:100	92	91

1. Extraction efficiency was determined by successive extractions using an optimized aqueous extraction buffer and analyzed by ELISA.
2. To evaluate the analytical accuracy of the ELISA, extracts prepared from each tissue type of conventional canola plants were spiked with known quantities of CP4 EPSPS protein at three concentrations spanning the range of the standard curve.

1.2 Matrix Effects

Matrix Effects acceptance criteria = 70-130%.
No matrix interferences (non-specific binding) were noted when sample extracts were analyzed at matrix dilutions stated below.

Tissue	Minimal Dilution to Avoid Matrix Effects	Average Percent Recovery (%)
Leaf	1:20	109
Root	1:20	94
Forage	1:20	99
Grain	1:20	94

1.3 Parallelism

Parallelism exists meaning that the plant produced CP4 EPSPS protein is immunologically equivalent to the *e.coli* produced CP4 EPSPS protein standard.
Parallelism acceptance criteria = 70-130%.

Tissue	Average Percent Recovery (%)
Leaf	95-100
Root	100-117
Forage	92-104
Grain	85-100

2.0 Precision*

(*NOTE: This precision data was taken from Soybean CP4 EPSPS Validation BR-ME-1087-01 validation. The assay portion of this validation (antibodies, standard, buffers) is identical to the Canola assay and precision was not repeated for AG-ME-1362.)

Range of Quantitation: 0.456-14.6 ng/ml
Method for Curve Fit 4-parameter

Intra-Assay Precision Acceptance Criteria: ≤ 15%
Inter-Assay Precision Acceptance Criteria: ≤ 25%
Precision Profile Acceptance Criteria: Standards 1-5 ≤ 15%
Standard 6 ≤ 25%

Intra-Assay Precision[3]: 2.9%
Inter-Assay Precision[3]: 13%

3. The inter- and intra-assay precision were assessed by determining the CV of the concentration of CP4 EPSPS protein measured for the positive control sample from 21 independent ELISAs using one-way analysis of variance (ANOVA).

Precision Profile:

Standard Number	Concentration (ng/ml)	%CV (over 21 runs)
1	14.6	9.1
2	7.3	7.4
3	3.65	7.3
4	1.825	4.0
5	0.913	6.5
6	0.456	4.7

The total intra-assay precision based on the standard curve was calculated to be **6.5**%

3.0 Sensitivity

Limits of Quantitation[4] and Detection[5]:

Tissue Type	Dilution	LOD (ng/ml)	LOD (µg/g fwt)	LOQ (ng/ml)	LOQ (µg/g fwt)
Leaf	1:20	0.049	0.098	0.456	0.912
Root	1:20	0.298	0.596	0.456	0.912
Forage	1:20	0.139	0.278	0.456	0.912
Grain	1:20	0.403	0.806	0.456	0.912

4. The limit of detection (LOD) was calculated as the mean value plus three SD using the data generated with conventional sample extracts for each tissue type. The LOD value in "ng/ml" was converted to "µg/g fwt" using the respective dilution factor and tissue-to-buffer ratio.
5. The limit of quantitation (LOQ) was calculated based on the lowest standard concentration. The "ng/ml" value was converted to "µg/g fwt" using the respective dilution factor and tissue-to-buffer ratio.

4.0 Extraction Parameters[6]

Tissue Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf	1:100	1X TBA
Root	1:100	1X TBA
Forage	1:100	1X TBA
Grain	1:100	1X TBA

6. The total CP4 EPSPS protein was extracted from each tissue by adding the appropriate volume of Extraction Buffer, and shaking in a Harbil mixer. The extracted sample was clarified using a serum filter.

Summary of Changes to Report

Page Numbers in MSL 0023090	Explanation Footnotes	Changes
1	1	Added "Amended Report for MSL 0022681" to Study Title; Changed [redacted] qualifications from B.S. to M.S.; Added "Amended Report before "Completed On" and revised amended report completion date.
1	1	Changed MSL number to MSL0023090
4	1	Added "Draft Report Amendment Review" to list of phases and provided a note for the addition of the draft report amendment review.
5	1	Changed [redacted] qualifications from B.S. to M.S.
6	1	Changed the Report Number from MSL0022681 to MSL0023090. Added "Amended Report for MSL 0022681" to Study Title. Added Amended Final Report Completion Date.
7	1	Revised Table of Contents to reflect changes in format and pagination.
7	1	Added "Summary of Changes to Report" in Table Contents.
10	1	Grain and root protein levels were updated due to protocol amendment. The mean CP4 EPSPS level for grain was changed from 32 µg/g dwt to 27 µg/g dwt. The mean CP4 EPSPS range for root tissues was changed from 41 - 82 µg/g dwt to 38 - 82 µg/g dwt.
11	1	Added *Escherichia coli* to Section 3.1.3.
12	1	Added to Section 4.2.2 the statement: A copy of the draft SOP was archived with the study.
14	1	Updated to the current Microsoft Excel version in Section 4.6. Added Section 4.7 to describe the protocol amendment to reopen the study.

Continued on next page

1. Denoted language used to distinguish the amended report from the original report. Items listed in the Summary of Changes to Report table do not impact the conclusions of this study.

Summary of Changes to Report (continued)

Page Numbers in MSL 0023090	Explanation Footnotes	Changes
15	1	Grain and root protein levels were updated due to protocol amendment. The mean CP4 EPSPS level for grain was changed from 32 µg/g dwt to 27 µg/g dwt. The mean CP4 EPSPS range for root tissues was changed from 41 - 82 µg/g dwt to 38 - 82 µg/g dwt.
16	1	Table 1: Changed the values of MON 88302 Grain due to protocol amendment. For the µg/g fwt values the mean and (SD) were changed from 29 (10) to 25 (5.2). The range was change from 21-56 to 21-43. For the µg/g dwt values the mean and (SD) were changed from 32 (11) to 27 (5.6). The range was change from 22-62 to 22-46.
17	1	Table 1: Changed the values of MON 88302 Root-2 due to protocol amendment. For the µg/g fwt value the (SD) was changed from (2.9) to (3.3). For the µg/g dwt values the mean and (SD) were changed from 41 (13) to 38 (14). Changed the "n" value for Grain from 20 to 16. Changed the "n" value for Root-2 from 15 to 11 in footnote 2. Added a statement for why the "n" values for Grain and Root-2 were reduced.
22	1	Added Summary of Changes to Report.

1. Denoted language used to distinguish the amended report from the original report. Items listed in the Summary of Changes to Report table do not impact the conclusions of this study.