

Title

**Analyses of the Raw Agricultural Commodity of Soybean Event FG72 for HPPDW336 and
2mEPSPS Proteins. USA 2009.**

Author

Martha R. Poe

Test Guideline

None

Completed On

October 23, 2009

Sponsor

**Cynthia Stauffer
Regulatory Affairs
Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709**

Testing Facility

**Bayer CropScience
BioAnalytics
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
USA**

Study Identification

DQ09B003

Total Number of Pages 43




M-357840-01-1

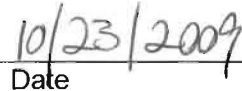
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Company Agent:


Isabelle Coats, Ph.D.
US Registration Manager
Regulatory Affairs - BioScience


Date

These data are the property of Bayer CropScience, and as such, are considered to be confidential for all purposes other than compliance with FIFRA § 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.

The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.


GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The undersigned hereby declare that the work to which this report refers was performed according to the procedures herein described and this report provides an accurate record of the results obtained. The study was conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160 except for the following:

1. The seed used in the study were not grown or produced under Good Laboratory Practice Standards.
2. The COA to characterize the test system was not conducted under GLP.
3. Samples were ground before there was an approved protocol.


None of these deviations has a negative impact on this study.

Study Director


Martha R. Poe
Scientist, BioAnalytics

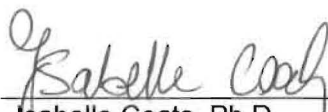
23 Oct. 2009
Date

Sponsor


Cynthia Stauffer
Regulatory Affairs - BioScience

23 Oct 2009
Date

Submitter

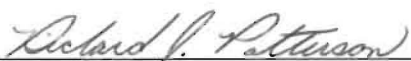

Isabelle Coats, Ph.D.
US Registration Manager
Regulatory Affairs - BioScience

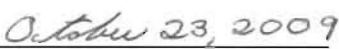
23 Oct. 2009
Date

QUALITY ASSURANCE STATEMENT OF INSPECTIONS

Study DQ09B003 was inspected by the Quality Assurance Unit for compliance with 40 CFR Part 160, Good Laboratory Practice Standards, according to Bayer CropScience Standard Operating Procedures. Following are the phases inspected, the dates inspections were completed and reported.

Dates Inspections Concluded	Phases Inspected	Dates Reported to Study Director	Dates Reported to Study Director Management
27 March 2009	Protocol	27 March 2009	27 March 2009
28 July 2009	ELISA	17 August 2009	17 August 2009
29 Sept 2009	Data and Records	29 Sept 2009	29 Sept 2009
29 Sept 2009	Draft Study Report	29 Sept 2009	29 Sept 2009
20 October 2009	Final Study Report	20 October 2009	20 October 2009


Richard J. Patterson, PhD
Quality Assurance Consultant


Date

APPROVALS PAGE

Study Director/Author



Martha R. Poe
Scientist
BioAnalytics - RTP
Bayer CropScience USA LP

23 Oct. 2009

Date

Sponsor

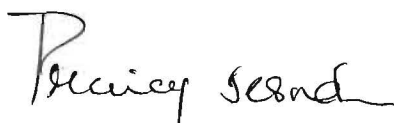


Cynthia Stauffer
Regulatory Affairs
Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
Phone: (919) 549-2856

23 Oct 2009

Date

Management



Princy Jesudason, Ph.D.
Manager, M.C./ BioAnalytics - RTP
Bayer CropScience USA LP
2 T.W. Alexander Drive
Research Triangle Park, NC 27709
Phone: (919) 549-2060

23 Oct 2009

Date

TABLE OF CONTENTS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS.....	2
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	3
QUALITY ASSURANCE STATEMENT OF INSPECTIONS	4
APPROVALS PAGE	5
TABLE OF CONTENTS	6
SUMMARY	8
STUDY IDENTIFICATION	9
1.0 INTRODUCTION.....	11
2.0 FIELD PHASE.....	11
Table 1: FG72 Soybean Field Trial Information from Study HT08SOY002	11
Table 2: FG72 Soybean Field Trial HT08SOY002 Regimens used for RAC Analysis.....	12
3.0 ANALYTICAL PHASE	12
3.1 Reference Substances	12
Table 3 Reference Substances	12
3.2 Certificates of Analysis	12
3.3 Samples	13
3.4 Sample Preparation	13
3.5 Extraction	13
3.6 ELISA Analyses	13
3.7 Standards.....	14
3.8 Validation	14
Table 4: Validation of Sample Extraction with Fortified NT Soybean Grain (BTID1754A).....	14
3.9 Limit of Detection and Limit of Quantification	14
Table 5: Limits of Detection and Quantification of 2mEPSPS and HPPDW336 in Fortified Non-transgenic Soybean Grain (BTID 1754A)	15
3.10 Protein analyte content.....	15
3.11 Statistical analysis	16
4.0 RESULTS AND DISCUSSION	16
Table 6. 2mEPSPS Protein in Unsprayed FG72 Soybean Grain.....	17
Table 7. 2mEPSPS Protein in Sprayed FG72 Soybean Grain.....	18
Table 8. HPPDW336 Protein in Unsprayed FG72 Soybean Grain	19
Table 9. HPPDW336 Protein in Sprayed FG72 Soybean Grain	20
Table 10: Amounts of 2mEPSPS and HPPDW336 in FG72 Soybean Grain	21
5.0 CONCLUSION	21
6.0 ARCHIVING	21

7.0	REFERENCES.....	22
APPENDIX 1	Certificate of Analysis	23
APPENDIX 2	Supporting Data	24
Table A2-1.	Critical Dates for Validation of 2mEPSPS and HPPDW336 Detection	25
Table A2-2	Critical Dates for FG72 Soybean Grain Analysis – Trial Sites 01-05	26
Table A2-3.	Critical Dates for FG72 Soybean Grain Analysis – Trial Sites 06-10	27
Table A2-4	Validation of 2mEPSPS ELISA for Soybean Grain using Sample BTID 1754A.....	28
Table A2-5	Validation of HPPD W336 ELISA for Soybean Grain using Sample BTID 1754A.	29
Table A2-6	Determination of the LOD for 2mEPSPS Protein in Non-transgenic Grain using Sample BTID 1754A	30
Table A2-7	Determination of the LOD for HPPD W336 Protein in Non-transgenic Grain using Sample BTID 1754A	30
Table A2-8	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 01.....	31
Table A2-9	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 02.....	31
Table A2-10	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 03.....	31
Table A2-11	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 04.....	32
Table A2-12	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 05.....	32
Table A2-13	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 06.....	32
Table A2-14	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 07.....	33
Table A2-15	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 08.....	33
Table A2-16	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 09.....	33
Table A2-17	Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 10.....	34
Table A2-18	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 01.....	34
Table A2-19	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 02.....	34
Table A2-20	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 03.....	35
Table A2-21	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 04.....	35
Table A2-22	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 05.....	35
Table A2-23	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 06.....	36
Table A2-24	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 07.....	36
Table A2-25	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 08.....	36
Table A2-26	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 09.....	37
Table A2-27	Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 10.....	37

SUMMARY

Analyses of the Raw Agricultural Commodity of Soybean Event FG72 for HPPDW336 and 2mEPSPS proteins. USA. 2009.

The transgenic soybean event FG72 contains the *hppdpfw336* and *2mepsps* genes which, when expressed in the plant, produce the HPPDW36 protein (hydroxyl phenyl-pyruvate-dioxygenase) and the 2mEPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase), respectively. The expression of the HPPDW36 protein confers tolerance to isoxaflutole herbicide and the expression of the 2mEPSPS protein confers tolerance to glyphosate herbicide. This study was carried out to determine the content of HPPDW336 and 2mEPSPS proteins in grain harvested from transgenic FG72 soybean.

Plants were grown at ten trial sites under conditions typical of production practices. There were six plots with event FG72 soybean at each test site. Three of the six transgenic event plots in each field trial were sprayed one time with the test herbicide Isoxaflutole + Glyphosate (IFT + GLY). The other three plots were untreated.

Samples of soybean seed were collected, stored at ambient temperatures, and shipped ambient to Bayer CropScience, Research Triangle Park, North Carolina. Within one week of arrival at Bayer CropScience, the seed samples were sub-sampled and transferred to frozen storage. Determination of the content of HPPDW336 and 2mEPSPS proteins in the raw agricultural commodity was accomplished via the use of protein-specific ELISAs.

The range and overall mean \pm standard deviation in ng/g for the fresh weight protein analyte content in grain samples from the 10 sites of transgenic soybean is presented in the table below. These numbers are taken from 12 individual results per regimen.

Dry weight and % crude protein analyte amounts were determined using the average of four individual results per sample, therefore no standard deviation or range is given for these amounts.

Protein	Treatment		Fresh Weight (ng/g)	Dry Weight (ng/g)	% Crude Protein
2mEPSPS	Unsprayed	Range	364 – 5790		
		Mean \pm SD	1360 \pm 1080	1500	0.00040
2mEPSPS	Sprayed	Range	326 – 2690		
		Mean \pm SD	1180 \pm 589	1300	0.00034
HPPDW336	Unsprayed	Range	455 – 1320		
		Mean \pm SD	846 \pm 183	936	0.00024
HPPDW336	Sprayed	Range	411 – 1313		
		Mean \pm SD	802 \pm 207	887	0.00023

STUDY IDENTIFICATION

Study Initiated:	July 17, 2009
Experimental Start Date:	July 21, 2009
Experimental Termination Date:	July 31, 2009
Study Completion Date:	October 23, 2009
Sponsor Facility Address:	Bayer CropScience 2 T. W. Alexander Drive Research Triangle Park, NC 27709
Personnel Involved with the Study:	Martha R. Poe Tonya Robinson Candice G. Matthews Shiney Isaac

ABBREVIATIONS, ACRONYMS and SCIENTIFIC TERMS

2mEPSPS - Modified 5-enolpyruvyl-shikimate-3-phosphate synthase

BSA – Bovine Serum Albumin

COA – Certificate of Analysis

COAs – Certificates of Analysis

CV – Coefficient of Variation

d.w. – dry weight

ELISA – Enzyme Linked ImmunoSorbent Assay

f.w. – fresh weight

g – gram

GLP – Good Laboratory Practice

GLY – glyphosate

HPPDW336 – Modified hydroxyl phenyl-pyruvate-dioxygenase

IFT – isoxaflutole

kDa – kilo Daltons

LOD – Limit of Detection

LOQ – Limit of Quantification

mL – milliLiter

ng – nanogram

NT – Non-transgenic

OD – Optical Density

RAC – Raw Agricultural Commodity

SD – Standard Deviation

1.0 INTRODUCTION

The transgenic soybean event FG72 contains the *hppdpfw336* and *2mepsps* genes which, when expressed in the plant, produce the HPPDW36 protein (hydroxyl phenyl-pyruvate-dioxygenase) and the 2mEPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase), respectively. The expression of the HPPDW36 protein confers tolerance to isoxaflutole herbicide and the expression of the 2mEPSPS protein confers tolerance to glyphosate herbicide. Planting double-herbicide-tolerant soybean varieties containing transformation event FG72 provides growers with new options for weed control using IFT herbicide (registered in North America as Balance Pro®) in combination with glyphosate herbicide.

Soybean and soybean processed products are used for animal feed and human consumption. As a consequence, data are required on the amount of 2mEPSPS and HPPDW336 proteins in raw agricultural commodities to support the food and feed safety assessment of event FG72 soybean.

This study was designed to provide the required data from the 2008 growing season in the United States. The field trials supplying samples for this study were performed under MS Technologies LLC BioTech Study ID Number HT08SOY002¹. The test system was selected because the target crop, cultural practices, and application techniques are all representative of the intended use pattern of the FG72 transgenic event soybean plants. The seed for all soybean grown, analyzed, and reported herein was supplied by MS Technologies LLC, Adel, Iowa.

2.0 FIELD PHASE

The transgenic event FG72 soybean (six plots), its non-transgenic counterpart (three plots), and three commercial, non-transgenic soybean lines (one plot of each) were grown successfully at 10 trial sites under Study Number HT08SOY002 as outlined in Table 1 below.

Table 1: FG72 Soybean Field Trial Information from Study HT08SOY002

Trial Number	Location	State	County	Principal Field Investigator
01	Marcus	IA	Cherokee	Justin Mason
02	Iowa Falls	IA	Hardin	Justin Mason
03	Scranton / Glidden	IA	Carroll	Justin Mason
04	Perry	IA	Dallas	Justin Mason
05	Adel	IA	Dallas	Justin Mason
06	Winterset	IA	Madison	Justin Mason
07	Osborn	MO	Clinton	Justin Mason
08	Fithian	IL	Vermillion	Jim Gappens
09	Sharpsville	IN	Tipton	Jim Gappens
10	Mediapolis	IN	Boone	Jim Gappens

There were a total of six regimens (labeled A-F) per trial as shown in the following table. The Jack and FG72 regimens were replicated three times while the commercial lines had only a single replicate within each trial, for a total of 12 soybean plots per trial.

Table 2: FG72 Soybean Field Trial HT08SOY002 Regimens used for RAC Analysis

Regi-men	Description	Matrix	Samples per Regimen	No. of Trials	Total Samples per Regimen
A	Jack: Non-transgenic Control ¹	Seed	3	10	30
B	FG72: Unsprayed Transgenic Plot	Seed	3	10	30
C	FG72: Sprayed Transgenic Plot	Seed	3	10	30

¹ Non-transgenic samples were not be analyzed for gene product content, so the total number of samples analyzed in this study was 60. However, a select sample was used as a negative control and for assay validation.

Only the transgenic soybean grain from regimens B and C were analyzed for gene product content, so the total number of samples analyzed in this study was 60. Regimen C received one foliar tank mix application of Balance[®] Pro (active ingredient: isoxaflutole) at 0.062 lb ai/A (70 g ai/ha), Roundup Original Max[®] (active ingredient: glyphosate) at 0.95 lb ai/A (1060 g ai/ha), and ammonium sulfate at 8 lb /100 gal (2850 g/ha) at post V4-V5 growth stage.

The soybean plots were harvested at normal maturity by hand or mechanical means to obtain the required seed RAC. The samples were stored at ambient temperatures after harvest and shipped ambient to the Bayer CropScience BioAnalytics department at Research Triangle Park (RTP), NC. The soybean seed samples were placed in frozen storage within one week of their arrival at RTP.

3.0 ANALYTICAL PHASE

3.1 Reference Substances

The reference substances used in this study are described in Table 3. 2mEPSPS and HPPDW336 protein reference substances were used as standards and to fortify non-transgenic sample HT08SOY002-08-11 (BTID 1754A). This non-transgenic sample was used for validation and recovery experiments.

Table 3 Reference Substances

Reference Substance (protein)	Batch No. / COA No.	Chemical name	Molecular Weight
2mEPSPS	LEJ5838 / BBS06-009 ²	modified 5-enolpyruvyl-shikimate-3-phosphate synthase	47 kDa ³
HPPDW336	LB020309 / BBS09-001 ⁴	modified hydroxyl phenyl-pyruvate-dioxygenase	40 kDa ⁵

3.2 Certificates of Analysis

The certificate of analysis (COA) No. BTS-0004/09 documenting the origin and identity of the samples harvested from the 10 trial sites of study HT08SOY002 by DNA analysis was

prepared by Bayer CropScience at Research Triangle Park, NC. Bayer CropScience in RTP analyzed the seed for the presence of DNA from event FG72.

The DNA analysis revealed that one conventional sample, BTID 1748C from Regimen A, contained event FG72 DNA. A sample from Regimen C at the same site should have been positive for event FG72 DNA, but did not contain event FG72 DNA. The principal field investigator was asked to provide more of each from the backup samples that had been taken. The backup samples were received and given BTID numbers 1846A and 1846B. The conventional soybean, BTID 1846A, did not contain event FG72 DNA; whereas, the transgenic soybean, BTID 1846B, did contain event FG72 DNA. This is the expected result, showing that the original samples were harvested from the correct plots, and that the backup samples were labeled correctly. This transgenic sample (BTID1846B) was used in the RAC analysis and in the composition study (DQ09B008) from which the moisture value was obtained.

Bayer CropScience in Gent, Belgium, produced reports serving as COAs for the 2mEPSPS and HPPDW336 proteins. A copy of each of the certificates of analysis is maintained with the study file.

3.3 Samples

The soybean grain samples were maintained at Bayer CropScience in Research Triangle Park, NC in a freezer at -10°C or lower until sample preparation. The amounts of 2mEPSPS and HPPDW336 proteins were determined at Bayer CropScience, NC. The critical dates for receipt, sample transfer to RTP, processing and analysis of the samples are given in Appendix 2, Tables A2-1 through A2-3.

3.4 Sample Preparation

Grain samples were ground in a blender pre-chilled with dry ice according to SOP 98034. Small amounts of dry ice were added to the blender periodically to ensure the samples remained frozen during preparation. A separate blender was used for each sample. The ground samples were stored in a freezer at approximately -20°C for overnight or longer to allow the dry ice to sublimate before extraction.

3.5 Extraction

Total protein was extracted from the raw agricultural product of soybean grain according to SOP 98033. PBST Extraction/Dilution Buffer, Agdia, Inc. Catalog Number ACC 00501A, was used. A representative fraction (approximately 0.1 g) of ground sample was mixed with 4 mL extraction buffer in a 50 mL polypropylene centrifuge tube, and then shaken for 30 minutes at ~ 4°C on a shaker (IKA-SCHÜTTLER MTS 4) at 250 rpm. The liquid extract was transferred to a clean centrifuge tube and centrifuged at approximately 18000 x g for 10 minutes at ~ 4°C. The clear supernatant was then used for 2mEPSPS and HPPDW336 analyses. Duplicate extracts were prepared for each sample. Total extractable protein was determined as a relative measure of extraction efficiency.

3.6 ELISA Analyses

The levels of HPPDW336 and 2mEPSPS proteins were determined by an Enzyme Linked ImmunoSorbent Assay (ELISA) using antibodies specific for each protein according to SOPs 98060 and 98052. SOP 98052 was modified by changing the extraction/dilution buffer to

PBST Extraction/Dilution Buffer, (Agdia, Inc. Catalog Number ACC 00501), and the sample to buffer ratio was 0.1g:4mL. Each ELISA assay was validated in soybean grain as described in the respective SOPs. All quantitative determinations of 2mEPSPS and HPPDW336 proteins were conducted at Bayer CropScience, Research Triangle Park, NC.

3.7 Standards

Protein standards were included in duplicate on each 2mEPSPS ELISA plate at the following concentrations: 32, 16, 8, 4, 2, 1, 0.5 ng/mL. The standards were plated in duplicate at concentrations of 32, 16, 8, 4, 2, 1 ng/mL on the HPPDW336 ELISA plate.

3.8 Validation

The ELISA procedures were validated with a non-transgenic sample, (Jack, Sample Number HT08SOY002-08-11 [BTID 1754A]) that was also produced in study HT08SOY002, using the protein standards described in Table 3 and SOP 98007. The critical dates for validation are listed in 110Appendix 2, Table A2-1. The non-transgenic sample was fortified at the concentrations listed in Table 4. The standards were added to the extraction buffer at the indicated concentrations prior to extraction in five replicates. Each replicate was analyzed using duplicate wells. A summary of the validation data for each analyte is shown in Table 4. The complete data sets are given in Appendix 2, Tables A2-4 and A2-5.

Table 4: Validation of Sample Extraction with Fortified NT Soybean Grain (BTID1754A)

2mEPSPS ELISA Validation				HPPDW336 ELISA Validation			
2mEPSPS fortified (ng/mL)	2mEPSPS detected (ng/mL) ^a Mean ± SD	% 2mEPSPS Recovery Mean ± SD	2mEPSPS Recovery %CV	HPPDW336 fortified (ng/mL)	HPPDW336 detected (ng/mL) ^a Mean ± SD	% HPPDW336 Recovery Mean ± SD	HPPDW336 Recovery %CV
100	82.5 ± 2.7	82.5 ± 2.7	3.32	100	123 ± 4	123 ± 4	3.01
32	23.0 ± 0.5	71.7 ± 1.6	2.21	32	31.4 ± 1.3	98.2 ± 3.9	4.01
16	10.4 ± 0.2	64.8 ± 1.6	2.45	16	18.0 ± 0.9	112 ± 6	5.26
8	5.14 ± 0.20	64.2 ± 2.6	3.98	8	8.34 ± 0.54	104 ± 7	6.47
4	2.59 ± 0.19	64.8 ± 4.7	7.23	4	3.51 ± 0.24	87.8 ± 6.0	6.78
2	1.21 ± 0.07	60.4 ± 3.5	5.74	2	1.43 ± 0.26	71.6 ± 13.1	18.2
1	0.62 ± 0.10	61.5 ± 9.7	15.7	1 ^b	0.43 ± 0.19	43.0 ± 19.2	44.6
0.5 ^b	0.45 ± 0.12	89.5 ± 23.2	26.0				

^a The protein analyte detected and its recovery are expressed as the average of 10 data points from duplicate extracts of 5 samples at each fortification level using non-transgenic matrix. Averages, standard deviations and %CV values are calculated with full precision and then rounded to 2 or 3 significant figures.

^b Validity criteria were not met at indicated concentration

3.9 Limit of Detection and Limit of Quantification

The limit of detection (LOD) is determined for the matrix using the average standard curve and the concentration derived from the background optical density (OD) of the negative control samples. The LOD is defined as the concentration corresponding to an OD value three standard deviations above the mean background OD.

The LOD is expressed in the unit of concentration (ng/mL) and the unit of weight ratio (ng/g matrix, i.e. ppb) calculated based on the extraction of an amount of the matrix with a known volume of extraction buffer, e.g., 0.1 g of matrix per 4 mL extraction buffer. The data used for these calculations are given in Appendix 2, Tables A2-6, A2-7 and are summarized in

Table 5 below. An absorbance reading giving rise to a protein analyte concentration above the LOD is assumed to be greater than the zero dose reading.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard that meets the validity criteria for the LOQ. Validity criteria are a) analyte recoveries from fortified matrix samples are $\geq 60\%$ and $\leq 130\%$ and b) the coefficient of variance (relative standard deviation) is less than 25%. When a lower recovery is caused by the nature of a specific matrix or the effect of a process, the lowest concentration of the standard that gives a coefficient of variance equal to or less than 25% is used as the LOQ. Values below the LOD are reported as zero and values below the LOQ but above the LOD are reported at the LOQ. In calculations, values below the LOD are treated as zero. Values below the LOQ but above the LOD are assumed to be at the LOQ. The LOQ values are determined by inspection from Tables A2-4 and A2-5. The estimated LOQ values are summarized in Table 5 below.

Table 5: Limits of Detection and Quantification of 2mEPSPS and HPPDW336 in Fortified Non-transgenic Soybean Grain (BTID 1754A).

Protein Analyte	LOD		LOQ	
2mEPSPS	0.26 ng/mL	10.3 ng/g ^a	1 ng/mL	40 ng/g ^a
HPPDW336	0.52 ng/mL	20.8 ng/g ^a	2 ng/mL	80 ng/g ^a

^a Calculated based on the extraction of 0.1 g matrix per 4 mL of extraction buffer.

3.10 Protein analyte content

SoftMax Pro™ software (Molecular Devices, Version 4.0) was used to derive the concentration of 2mEPSPS and HPPDW336 proteins from the ELISA data. Absorbance units were adjusted for the buffer blank. A set of wells containing samples of the corresponding non-transgenic matrix was included on each plate for background subtraction. The appropriate background corrections for the transgenic grain samples were obtained from background values of a non-transgenic grain sample, BTID 1754A, which was diluted on the same plate and to the same extent as the transgenic grain sample. Thus the dilution of the non-transgenic sample used for background subtraction was the same as the dilution of the transgenic sample that was required in order to place the OD reading in the center portion of the standard curve. The absorbance readings corrected for both buffer blank and non-transgenic background were converted to the protein concentration using the standard curve. The analytical results from the ELISA data are presented in Appendix 2, Tables A2-8 through A2-27.

The ELISA assays give results in units of ng of analyte per milliliter of extract that are then converted into ng of analyte per gram of fresh sample. As different tissues have different protein and water contents, the results are also expressed as percent of crude protein. Percent moisture and crude protein (fresh and dry weight basis) data for these samples were obtained from study DQ08B009⁶. Results presented are rounded to 2 or 3 significant figures.

Analyte as percent crude protein is obtained by dividing the fresh weight content of analyte by the % crude protein value, then dividing by 100000 for unit conversion, as shown in the following formula.

$$\text{analyte as \% crude protein} = \frac{\left(\frac{\text{ng analyte / g}}{\% \text{ crude protein}} \right)}{100000}$$

Dry weight analyte content is obtained by dividing the fresh weight analyte content by the calculated percent dry matter and multiplying by 100. Percent dry matter is obtained by subtracting the percent moisture from 100. The following is the formula used.

$$\text{analyte dry weight content ng / g} = \left(\frac{\text{ng analyte / g}}{(100 - \% \text{ moisture})} \right) \times 100$$

3.11 Statistical analysis

Descriptive statistics (mean, standard deviation, and coefficient of variance) were calculated for each sample matrix and treatment⁷. Bayer CropScience conducted all statistical analyses.

4.0 RESULTS AND DISCUSSION

Transgenic soybean grain samples were analyzed to determine the amounts of 2mEPSPS protein and HPPDW336 protein, and total crude protein. The amounts (fresh weight basis) of 2mEPSPS and HPPDW336 proteins in soybean grain from each site were determined by ELISA. The amounts of each analyte measured in soybean grain, as a proportion of dry weight, and as percent of crude protein are presented in Tables 6-9. Means and standard deviations reported are based on 12 measurements resulting from duplicate assays on duplicate extractions of samples from three replicates (3 replicates x 2 extractions x 2 assays). Values are rounded to 2 or 3 significant figures. A summary of the results is presented in Table 10.

In grain harvested from unsprayed soybean plants, 2mEPSPS protein content from the 10 trial sites ranged from 364 to 5790 ng/g, with an overall average of 1360 ng/g. The overall average 2mEPSPS protein content in grain harvested from sprayed soybean plants from the 10 sites was 1180 ng/g, with values ranging from 326 to 2690 ng/g. HPPDW336 protein content in grain from unsprayed plants ranged from 455 to 1320 ng/g, with an overall average of 846 ng/g. The amount of HPPDW336 in grain harvested from sprayed soybean plants ranged from 411 to 1313 ng/g, with an overall average of 802 ng/g.

On a dry weight basis, average 2mEPSPS protein content was 1500 ng/g. The overall average 2mEPSPS protein content in grain harvested from sprayed soybean plants was 1300 ng/g. Average HPPDW336 protein content in untreated grain was 936 ng/g. The overall average HPPDW336 protein content in grain harvested from sprayed soybean plants from the 10 sites was 887 ng/g.

The percent of crude protein and the amounts of analyte protein in soybean grain were used to calculate the percent of 2mEPSPS and HPPDW336 proteins present in unsprayed and sprayed soybean grain. The 2mEPSPS protein represented on average 0.00040 % of the total crude protein in unsprayed grain. In sprayed grain, the 2mEPSPS protein represented on average 0.00034 % of the total crude protein. The HPPDW336 protein represented on average 0.00024 % of the total crude protein in unsprayed grain. In sprayed grain, the HPPDW336 protein represented on average 0.00023 % of the total crude protein.

Table 6. 2mEPSPS Protein in Unsprayed FG72 Soybean Grain

Trial Site	BTID	2mEPSPS Content (fresh weight)		2mEPSPS Content (dry weight)			2mEPSPS Content as % Crude Protein		
		f.w. 2mEPSPS Content (ng/g)	f.w. Mean \pm SD (ng/g)	% Moisture	d.w. 2mEPSPS Content (ng/g)	d.w. Mean (ng/g)	% Crude Protein (f.w.)	2mEPSPS as % Crude Protein	Mean
01	1747D	840	713 \pm 124	10.1	934	794	34.9	0.00024	0.00021
	1747E	599		9.86	665		34.7	0.00017	
	1747F	701		10.4	782		34.3	0.00020	
02	1748D	707	660 \pm 171	11.5	799	743	34.4	0.00021	0.00019
	1748E	780		10.9	875		35.1	0.00022	
	1748F	493		11.2	555		35.3	0.00014	
03	1749D	688	573 \pm 162	10	764	636	34.8	0.00020	0.00017
	1749E	522		9.91	579		34.3	0.00015	
	1749F	510		9.83	566		34.6	0.00015	
04	1750D	553	769 \pm 216	9.07	608	845	33.7	0.00016	0.00023
	1750E	960		8.89	1,050		33.8	0.00028	
	1750F	793		9.36	875		33.7	0.00024	
05	1751D	627	610 \pm 111	9.51	692	674	35.3	0.00018	0.00018
	1751E	679		9.32	748		33.5	0.00020	
	1751F	524		9.86	582		34.8	0.00015	
06	1752D	854	886 \pm 194	10.1	950	984	34.8	0.00025	0.00025
	1752E	689		10.3	768		35.6	0.00019	
	1752F	1,120		9.58	1,230		35.2	0.00032	
07	1753D	1,230	1,260 \pm 227	9.62	1,360	1,400	33.3	0.00037	0.00037
	1753E	1,500		9.41	1,650		33.9	0.00044	
	1753F	1,060		9.79	1,170		34.2	0.00031	
08	1754D	1,760	2,310 \pm 557	9.8	1,950	2,570	34.6	0.00051	0.00068
	1754E	2,910		10.2	3,240		34.0	0.00086	
	1754F	2,250		10.2	2,500		33.7	0.00067	
09	1755D	1,790	2,290 \pm 490	7.9	1,940	2,490	35.6	0.00050	0.00065
	1755E	2,860		8.26	3,120		35.1	0.00081	
	1755F	2,220		8.18	2,410		35.2	0.00063	
10	1756D	3,050	3,570 \pm 1,290	8.65	3,340	3,920	34.6	0.00088	0.0010
	1756E	5,230		8.8	5,730		34.3	0.0015	
	1756F	2,430		8.93	2,670		33.9	0.00072	
		Mean \pm SD	1360 \pm 1080		Mean	1500		Mean	0.00040
		Range ^a	364 – 5790						

^aRange of 2mEPSPS fresh weight content from individual results.

Table 7. 2mEPSPS Protein in Sprayed FG72 Soybean Grain

Trial Site	BTID	2mEPSPS Content (fresh weight)		2mEPSPS Content (dry weight)			2mEPSPS Content as % Crude Protein		
		f.w. 2mEPSPS Content (ng/g)	f.w. Mean \pm SD (ng/g)	% Moisture	d.w. 2mEPSPS Content (ng/g)	d.w. Mean (ng/g)	% Crude Protein (f.w.)	2mEPSPS as % Crude Protein	Mean
01	1747G	754	844 \pm 147	10	838	940	34.4	0.00022	0.00025
	1747H	831		10.4	928		34.3	0.00024	
	1747I	946		10.2	1,050		34.5	0.00027	
02	1748G	508	702 \pm 337	10.9	570	778	34.3	0.00015	0.00020
	1846B	458		6.51	490		34.3	0.00013	
	1748I	1,140		10.5	1,270		35.4	0.00032	
03	1749G	695	601 \pm 111	10.3	775	668	34.7	0.00020	0.00017
	1749H	546		9.83	605		34.3	0.00016	
	1749I	562		9.79	623		34.8	0.00016	
04	1750G	1,250	1,010 \pm 235	9.15	1,380	1,110	33.7	0.00037	0.00030
	1750H	1,010		9.53	1,110		33.0	0.00031	
	1750I	763		9.66	845		34.1	0.00022	
05	1751G	929	971 \pm 181	8.94	1,020	1,070	34.3	0.00027	0.00028
	1751H	985		9.42	1,090		34.3	0.00029	
	1751I	1,000		9.05	1,100		34.3	0.00029	
06	1752G	665	842 \pm 199	9.83	738	930	34.9	0.00019	0.00024
	1752H	1,070		9.38	1,180		35.3	0.00030	
	1752I	790		9.37	872		35.9	0.00022	
07	1753G	621	1,190 \pm 598	9.23	684	1,310	33.2	0.00019	0.00035
	1753H	1,930		9.1	2,130		33.4	0.00058	
	1753I	1,010		9.45	1,120		33.9	0.00030	
08	1754G	1,970	2,130 \pm 498	10	2,190	2,360	34.5	0.00057	0.00062
	1754H	1,960		9.33	2,160		35.6	0.00055	
	1754I	2,450		10	2,730		33.1	0.00074	
09	1755G	2,160	1,840 \pm 607	8.57	2,360	2,010	34.5	0.00063	0.00052
	1755H	1,080		8.11	1,180		36.2	0.00030	
	1755I	2,270		8.5	2,480		35.6	0.00064	
10	1756G	2,100	1,670 \pm 331	9.67	2,320	1,850	34.8	0.00060	0.00048
	1756H	1,450		9.18	1,590		33.9	0.00043	
	1756I	1,470		9.71	1,630		35.1	0.00042	
		Mean \pm SD	1180 \pm 589		Mean	1300		Mean	0.00034
		Range ^a	326 – 2690						

^aRange of 2mEPSPS fresh weight content from individual results.

Table 8. HPPDW336 Protein in Unsprayed FG72 Soybean Grain

Trial Site	BTID	HPPD Content (fresh weight)		HPPD Content (dry weight)			HPPD Content as % Crude Protein		
		f.w. HPPD Content (ng/g)	f.w. Mean \pm SD (ng/g)	% Moisture	d.w. HPPD Content (ng/g)	d.w. Mean (ng/g)	% Crude Protein (f.w.)	HPPD as % Crude Protein	Mean
01	1747D	981	1,040 \pm 129	10.1	1,090	1,150	34.9	0.00028	0.00030
	1747E	1,000		9.86	1,110		34.7	0.00029	
	1747F	1,130		10.4	1,260		34.3	0.00033	
02	1748D	1,010	1,020 \pm 111	11.5	1,140	1,150	34.4	0.00029	0.00029
	1748E	1,090		10.9	1,220		35.1	0.00031	
	1748F	965		11.2	1,090		35.3	0.00027	
03	1749D	877	894 \pm 215	10	974	992	34.8	0.00025	0.00026
	1749E	710		9.91	788		34.3	0.00021	
	1749F	1,100		9.83	1,220		34.6	0.00032	
04	1750D	845	872 \pm 95	9.07	929	959	33.7	0.00025	0.00026
	1750E	950		8.89	1,040		33.8	0.00028	
	1750F	820		9.36	905		33.7	0.00024	
05	1751D	604	599 \pm 102	9.51	667	663	35.3	0.00017	0.00017
	1751E	564		9.32	622		33.5	0.00017	
	1751F	630		9.86	699		34.8	0.00018	
06	1752D	858	806 \pm 146	10.1	954	895	34.8	0.00025	0.00023
	1752E	623		10.3	694		35.6	0.00017	
	1752F	938		9.58	1,040		35.2	0.00027	
07	1753D	651	724 \pm 102	9.62	720	801	33.3	0.00020	0.00021
	1753E	830		9.41	916		33.9	0.00024	
	1753F	692		9.79	767		34.2	0.00020	
08	1754D	587	626 \pm 60	9.8	651	696	34.6	0.00017	0.00018
	1754E	611		10.2	681		34	0.00018	
	1754F	679		10.2	757		33.7	0.00020	
09	1755D	741	921 \pm 176	7.9	805	1,000	35.6	0.00021	0.00026
	1755E	1,080		8.26	1,180		35.1	0.00031	
	1755F	940		8.18	1,020		35.2	0.00027	
10	1756D	1,070	956 \pm 182	8.65	1,170	1,050	34.6	0.00031	0.00028
	1756E	1,080		8.8	1,180		34.3	0.00031	
	1756F	719		8.93	789		33.9	0.00021	
		Mean \pm SD	846 \pm 183		Mean	936		Mean	0.00024
		Range ^a	455 – 1320						

^aRange of HPPDW336 fresh weight content from individual results.

Table 9. HPPDW336 Protein in Sprayed FG72 Soybean Grain

Trial Site	BTID	HPPD Content (fresh weight)		HPPD Content (dry weight)			HPPD Content as % Crude Protein		
		f.w. HPPD Content (ng/g)	f.w. Mean \pm SD (ng/g)	% Moisture	d.w. HPPD Content (ng/g)	d.w. Mean (ng/g)	% Crude Protein (f.w.)	HPPD as % Crude Protein	Mean
01	1747G	1,260	1,170 \pm 208	10	1,400	1,310	34.4	0.00037	0.00034
	1747H	1,010		10.4	1,120		34.3	0.00029	
	1747I	1,250		10.2	1,400		34.5	0.00036	
02	1748G	778	841 \pm 286	10.9	873	932	34.3	0.00023	0.00024
	1846B	569		6.51	609		34.3	0.00017	
	1748I	1,180		10.5	1,320		35.4	0.00033	
03	1749G	970	947 \pm 58	10.3	1,080	1,050	34.7	0.00028	0.00027
	1749H	901		9.83	999		34.3	0.00026	
	1749I	969		9.79	1,070		34.8	0.00028	
04	1750G	707	875 \pm 158	9.15	778	967	33.7	0.00021	0.00026
	1750H	985		9.53	1,090		33	0.00030	
	1750I	934		9.66	1,030		34.1	0.00027	
05	1751G	699	674 \pm 112	8.94	768	742	34.3	0.00020	0.00020
	1751H	574		9.42	634		34.3	0.00017	
	1751I	749		9.05	823		34.3	0.00022	
06	1752G	676	759 \pm 152	9.83	750	839	34.9	0.00019	0.00021
	1752H	923		9.38	1,020		35.3	0.00026	
	1752I	679		9.37	749		35.9	0.00019	
07	1753G	708	695 \pm 108	9.23	780	766	33.2	0.00021	0.00021
	1753H	694		9.1	763		33.4	0.00021	
	1753I	683		9.45	754		33.9	0.00020	
08	1754G	486	572 \pm 101	10	540	634	34.5	0.00014	0.00017
	1754H	564		9.33	622		35.6	0.00016	
	1754I	666		10	740		33.1	0.00020	
09	1755G	1,010	827 \pm 172	8.57	1,100	903	34.5	0.00029	0.00023
	1755H	617		8.11	671		36.2	0.00017	
	1755I	857		8.5	937		35.6	0.00024	
10	1756G	596	656 \pm 60	9.67	660	725	34.8	0.00017	0.00019
	1756H	661		9.18	728		33.9	0.00019	
	1756I	712		9.71	788		35.1	0.00020	
		Mean \pm SD	802 \pm 207		Mean	887		Mean	0.00023
		Range ^a	411 – 1313						

^aRange of HPPDW336 fresh weight content from individual results.

The amount of each analyte on a fresh weight, dry weight, and % crude protein (f.w.) basis are summarized in Table 10

The range and mean of fresh weight analyte content (ng/g) shown in the table were determined from 12 individual results per regimen.

Dry weight and % crude protein analyte amounts were determined using the average of four individual results per sample, therefore no standard deviation or range is given for these amounts.

Table 10: Amounts of 2mEPSPS and HPPDW336 in FG72 Soybean Grain

Protein	Treatment		Fresh Weight (ng/g)	Dry Weight (ng/g)	% Crude Protein
2mEPSPS	Unsprayed	Range	364 – 5790		
		Mean \pm SD	1360 \pm 1080	1500	0.00040
2mEPSPS	Sprayed	Range	326 – 2690		
		Mean \pm SD	1180 \pm 589	1300	0.00034
HPPDW336	Unsprayed	Range	455 – 1320		
		Mean \pm SD	846 \pm 183	936	0.00024
HPPDW336	Sprayed	Range	411 – 1313		
		Mean \pm SD	802 \pm 207	887	0.00023

5.0 CONCLUSION

The 2mEPSPS and HPPDW336 proteins were detected in all transgenic soybean grain samples.. The results show that the expression of the 2mEPSPS and HPPDW336 proteins is similar between sprayed and unsprayed FG72 soybean plants.

6.0 ARCHIVING

The final report, raw data, computer generated listings of raw data and supporting documentation are archived under study number DQ09B003 in the Archives of Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709. Retained samples are stored at the same address.

7.0 REFERENCES

- | <u>No.</u> | <u>Author(s).</u> | <u>Year.</u> | <u>Title.</u> | <u>Source.</u> | <u>Edition.</u> | <u>Pages.</u> |
|------------|---|--------------|--|--|---------------------------------------|---------------|
| 1 | Kowite, William J. | 2009. | Production of Raw Agricultural Commodities (Grain) of Transgenic Event FG72 Soybeans from Multiple Field Trials, USA, 2008. | Study Identification Number HT08SOY002. | | |
| 2 | Bautsoens, N. | 2006. | Certificate of Analysis for the 2mEPSPS protein produced in <i>E. coli</i> . | Report Number BBS06-009. | | |
| 3 | Lebrun, M., Sailland, A., Freyssinet, G., and Degryse, E. | 1997. | Mutated 5-enolpyruvylshikimate-3-phosphate synthase, gene encoding for said protein and transformed plants containing said gene. | International patent publication WO 97/04103-A2. | 06.02.97. | 25 pages. |
| 4 | Habex, V. | 2009. | Certificate of Analysis for the HPPD W336 protein produced in <i>E. coli</i> batch n°LB020309. | Report Number BBS09-001. | | |
| 5 | Hendrickx, K. | 2009. | The mutant 4-hydroxyphenylpyruvate dioxygenase gene product:HPPD W336 description and characterization. | Report Identification Number BM99L213. | | |
| 6 | Mackie, Sandra J. W. | 2009. | Composition of Seed from FG72 Soybean and its Non-transgenic Counterpart. | USA. 2008. | Study Identification Number DQ08B009. | |
| 7 | Devore, J; Peck, R. | (1986). | Statistics. | West Publishing Company, St. Paul | | |

APPENDIX 1
Certificate of Analysis

CERTIFICATE OF ANALYSIS NO. BTS-0004/09

General

This Certificate fulfills the requirement for the characterization of seed and/or sample used in a study. It documents the identity and purity/content of the seed and/or sample.

Designation of the Certified Material:

Material: Event FG72 herbicide tolerant soybean raw agricultural commodity (RAC) and conventional soybean RAC

Study No.: HT08SOY002

Sample Nos.: Biotechnology Identification (BTID) numbers 1747A-L; 1748A-L; 1749A-L; 1750A-L; 1751A-L; 1752A-L; 1753A-L; 1754A-L; 1755A-L; 1756A-L and 1846A-B

Additional information: RAC analyzed in composition study DQ08B009 and in GPC study DQ09B003

Origin of the Certified Material

RAC samples were obtained from the ten sites included in study HT08SOY002:

Trial Number	Location	State	County	Principal Field Investigator
01	Marcus	IA	Cherokee	Justin Mason
02	Iowa Falls	IA	Hardin	Justin Mason
03	Scranton / Glidden	IA	Carroll	Justin Mason
04	Perry	IA	Dallas	Justin Mason
05	Adel	IA	Dallas	Justin Mason
06	Winterset	IA	Madison	Justin Mason
07	Osborn	MO	Clinton	Justin Mason
08	Fithian	IL	Vermillion	Jim Gappens
09	Sharpville	IN	Tipton	Jim Gappens
10	Mediapolis	IN	Boone	Jim Gappens

Methods

The ☒ Identity
☐ Purity
☐ Content

of the material was established by use of the following method(s):

- ☐ PCR for
☐ *bar* ☐ *pat* ☐ *cry1Ab* ☐ *cry2Ae* ☐ other _____
- ☐ Discriminating PCR
- ☐ Southern Blotting
- ☐ ELISA
- ☐ Bradford Assay
- ☒ Real-Time PCR (Methods PGS0475, MDP0678)
- ☐ Lateral Flow Strip Test

This COA was produced according to SOP 98003.01.

Dates of Analysis

December 9, 2008 to March 6, 2009

Methods and Results

Five soybeans were ground together in a small vial using a Genogrinder. DNA was extracted from approximately 150mg of the crushed soybean using a silica gel based technology. DNA extracts were analyzed by Real-Time Discriminating PCR (RTdPCR) for event FG72 and a soybean reference gene.

All soybean RAC extracts were analyzed for the presence of event FG72 DNA. One conventional sample, BTID 1748C from Regimen A, was shown to contain event FG72 DNA. A sample from Regimen C at the same site should have been positive for event FG72 DNA, but did not contain event FG72 DNA. This result was confirmed for both samples by analyzing a second 5-seed batch from both sample bags. The principal field investigator was asked to provide more of each from the backup samples that had been taken.

The backup samples were received and given BTID numbers 1846A and 1846B. A 5-seed sample of each was analyzed for the presence of event FG72 DNA. The conventional soybean, BTID 1846A, did not contain event FG72 DNA; whereas, the transgenic soybean, BTID 1846B, did contain event FG72 DNA. This is the expected result, showing that the original samples were harvested from the correct plots, and that the backup samples were labeled correctly. These samples (BTID1846A-B) will be used in subsequent composition and GPC analyses.

All other conventional soybean DNA extracts produced only the endogenous PCR product, confirming that these samples were harvested from non-transgenic plots. All other transgenic soybean RAC samples contain event FG72 DNA, confirming that they were harvested from the transgenic plots.

Table 3 lists BTID numbers and corresponding field sample numbers for sample identification, as well as FG72 RTdPCR results.

TABLE 3 PCR Results, BTID Numbers, and Field Sample Numbers

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1747A	HT08SOY002-01-11	-
	1747B	HT08SOY002-01-12	-
	1747C	HT08SOY002-01-13	-
B	1747D	HT08SOY002-01-21	+
	1747E	HT08SOY002-01-22	+
	1747F	HT08SOY002-01-23	+
C	1747G	HT08SOY002-01-31	+
	1747H	HT08SOY002-01-32	+
	1747I	HT08SOY002-01-33	+
D	1747J	HT08SOY002-01-41	-
E	1747K	HT08SOY002-01-42	-
F	1747L	HT08SOY002-01-43	-

TABLE 3 PCR Results, BTID Numbers, and Field Sample Numbers

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1748A	HT08SOY002-02-11	-
	1748B	HT08SOY002-02-12	-
	1748C ^a	HT08SOY002-02-13	+
	1846A	HT08SOY002-02-13	-
B	1748D	HT08SOY002-02-21	+
	1748E	HT08SOY002-02-22	+
	1748F	HT08SOY002-02-23	+
C	1748G	HT08SOY002-02-31	+
	1748H ^b	HT08SOY002-02-32	-
	1846B	HT08SOY002-02-32	+
	1748I	HT08SOY002-02-33	+
D	1748J	HT08SOY002-02-41	-
E	1748K	HT08SOY002-02-42	-
F	1748L	HT08SOY002-02-43	-

^aBTID 1748C was replaced with BTID1846A in subsequent analyses.

^bBTID 1748H was replaced with BTID 1846B in subsequent analyses.

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1749A	HT08SOY002-03-11	-
	1749B	HT08SOY002-03-12	-
	1749C	HT08SOY002-03-13	-
B	1749D	HT08SOY002-03-21	+
	1749E	HT08SOY002-03-22	+
	1749F	HT08SOY002-03-23	+
C	1749G	HT08SOY002-03-31	+
	1749H	HT08SOY002-03-32	+
	1749I	HT08SOY002-03-33	+
D	1749J	HT08SOY002-03-41	-
E	1749K	HT08SOY002-03-42	-
F	1749L	HT08SOY002-03-43	-

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1750A	HT08SOY002-04-11	-
	1750B	HT08SOY002-04-12	-
	1750C	HT08SOY002-04-13	-
B	1750D	HT08SOY002-04-21	+
	1750E	HT08SOY002-04-22	+
	1750F	HT08SOY002-04-23	+
C	1750G	HT08SOY002-04-31	+
	1750H	HT08SOY002-04-32	+
	1750I	HT08SOY002-04-33	+
D	1750J	HT08SOY002-04-41	-
E	1750K	HT08SOY002-04-42	-
F	1750L	HT08SOY002-04-43	-

TABLE 3 PCR Results, BTID Numbers, and Field Sample Numbers

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1751A	HT08SOY002-05-11	-
	1751B	HT08SOY002-05-12	-
	1751C	HT08SOY002-05-13	-
B	1751D	HT08SOY002-05-21	+
	1751E	HT08SOY002-05-22	+
	1751F	HT08SOY002-05-23	+
C	1751G	HT08SOY002-05-31	+
	1751H	HT08SOY002-05-32	+
	1751I	HT08SOY002-05-33	+
D	1751J	HT08SOY002-05-41	-
E	1751K	HT08SOY002-05-42	-
F	1751L	HT08SOY002-05-43	-

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1752A	HT08SOY002-06-11	-
	1752B	HT08SOY002-06-12	-
	1752C	HT08SOY002-06-13	-
B	1752D	HT08SOY002-06-21	+
	1752E	HT08SOY002-06-22	+
	1752F	HT08SOY002-06-23	+
C	1752G	HT08SOY002-06-31	+
	1752H	HT08SOY002-06-32	+
	1752I	HT08SOY002-06-33	+
D	1752J	HT08SOY002-06-41	-
E	1752K	HT08SOY002-06-42	-
F	1752L	HT08SOY002-06-43	-

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 result</i>
A	1753A	HT08SOY002-07-11	-
	1753B	HT08SOY002-07-12	-
	1753C	HT08SOY002-07-13	-
B	1753D	HT08SOY002-07-21	+
	1753E	HT08SOY002-07-22	+
	1753F	HT08SOY002-07-23	+
C	1753G	HT08SOY002-07-31	+
	1753H	HT08SOY002-07-32	+
	1753I	HT08SOY002-07-33	+
D	1753J	HT08SOY002-07-41	-
E	1753K	HT08SOY002-07-42	-
F	1753L	HT08SOY002-07-43	-

TABLE 3 PCR Results, BTID Numbers, and Field Sample Numbers

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 PCR result</i>
A	1754A	HT08SOY002-08-11	-
	1754B	HT08SOY002-08-12	-
	1754C	HT08SOY002-08-13	-
B	1754D	HT08SOY002-08-21	+
	1754E	HT08SOY002-08-22	+
	1754F	HT08SOY002-08-23	+
C	1754G	HT08SOY002-08-31	+
	1754H	HT08SOY002-08-32	+
	1754I	HT08SOY002-08-33	+
D	1754J	HT08SOY002-08-41	-
E	1754K	HT08SOY002-08-42	-
F	1754L	HT08SOY002-08-43	-

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 PCR result</i>
A	1755A	HT08SOY002-09-11	-
	1755B	HT08SOY002-09-12	-
	1755C	HT08SOY002-09-13	-
B	1755D	HT08SOY002-09-21	+
	1755E	HT08SOY002-09-22	+
	1755F	HT08SOY002-09-23	+
C	1755G	HT08SOY002-09-31	+
	1755H	HT08SOY002-09-32	+
	1755I	HT08SOY002-09-33	+
D	1755J	HT08SOY002-09-41	-
E	1755K	HT08SOY002-09-42	-
F	1755L	HT08SOY002-09-43	-

<i>Regimen</i>	<i>BTID:</i>	<i>Field Sample Number</i>	<i>FG72 PCR result</i>
A	1756A	HT08SOY002-10-11	-
	1756B	HT08SOY002-10-12	-
	1756C	HT08SOY002-10-13	-
B	1756D	HT08SOY002-10-21	+
	1756E	HT08SOY002-10-22	+
	1756F	HT08SOY002-10-23	+
C	1756G	HT08SOY002-10-31	+
	1756H	HT08SOY002-10-32	+
	1756I	HT08SOY002-10-33	+
D	1756J	HT08SOY002-10-41	-
E	1756K	HT08SOY002-10-42	-
F	1756L	HT08SOY002-10-43	-

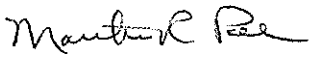
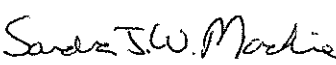
BioAnalytics
Bayer CropScience
BioScience
2 TW Alexander Drive
RTP, NC 27709
USA

No. BTS-0004/09
Page 6 of 6

Testing Facility

BioAnalytics
Bayer CropScience
BioScience
2 T.W. Alexander Dr.
Research Triangle Park, NC 27709
USA

Report and raw data are archived at Bayer CropScience, RTP, NC.

	Name (Typed)	Signature	Date (M/D/Y)
Responsible Scientist:	Martha R. Poe		March 25, 2009
Authorized by:	Sandra J.W. Mackie		March 25, 2009

APPENDIX 2
Supporting Data

Table A2-1. Critical Dates for Validation of 2mEPSPS and HPPDW336 Detection

Sample ID:	Matrix	Sample Received	Sample Ground	Sample Extracted	2mEPSPS Assayed	HPPDW336 Assayed
1754A	Grain	11/13/2008	4/3/2009	7/21/2009	7/21/2009	7/21/2009

Table A2-2 Critical Dates for FG72 Soybean Grain Analysis – Trial Sites 01-05

Trial Site	Regimen	BTID	Sample ID	Replicate	Sample Received	Sample ground	2mEPSPS Analysis		HPPDW336 Analysis	
							Sample extracted	2mEPSPS assayed	Sample extracted	HPPD assayed
01	B	1747D	HT08SOY002-01-21	1	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1747E	HT08SOY002-01-22	2	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1747F	HT08SOY002-01-23	3	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
	C	1747G	HT08SOY002-01-31	1	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1747H	HT08SOY002-01-32	2	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1747I	HT08SOY002-01-33	3	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
02	B	1748D	HT08SOY002-02-21	1	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1748E	HT08SOY002-02-22	2	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1748F	HT08SOY002-02-23	3	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
	C	1748G	HT08SOY002-02-31	1	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1846B	HT08SOY002-02-32	2	11/13/2008	4/8/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1748I	HT08SOY002-02-33	3	11/13/2008	4/6/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
03	B	1749D	HT08SOY002-03-21	1	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1749E	HT08SOY002-03-22	2	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1749F	HT08SOY002-03-23	3	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
	C	1749G	HT08SOY002-03-31	1	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1749H	HT08SOY002-03-32	2	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1749I	HT08SOY002-03-33	3	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
04	B	1750D	HT08SOY002-04-21	1	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1750E	HT08SOY002-04-22	2	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
		1750F	HT08SOY002-04-23	3	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
	C	1750G	HT08SOY002-04-31	1	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1750H	HT08SOY002-04-32	2	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/23/2009	7/23/2009
		1750I	HT08SOY002-04-33	3	11/13/2008	4/7/2009	7/23/2009	7/23/2009	7/24/2009	7/24/2009
05	B	1751D	HT08SOY002-05-21	1	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1751E	HT08SOY002-05-22	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1751F	HT08SOY002-05-23	3	11/13/2008	4/7/2009	7/31/2009	7/31/2009	7/27/2009	7/27/2009
	C	1751G	HT08SOY002-05-31	1	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1751H	HT08SOY002-05-32	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1751I	HT08SOY002-05-33	3	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009

Regimen: B = Unsprayed; C = Sprayed with IFT + GLY herbicide

Table A2-3. Critical Dates for FG72 Soybean Grain Analysis – Trial Sites 06-10

Trial Site	Regimen	BTID	Sample ID	Replicate	Sample Received	Sample ground	2mEPSPS Analysis		HPPDW336 Analysis	
							Sample extracted	2mEPSPS assayed	Sample extracted	HPPD assayed
06	B	1752D	HT08SOY002-06-21	1	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1752E	HT08SOY002-06-22	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1752F	HT08SOY002-06-23	3	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
	C	1752G	HT08SOY002-06-31	1	11/13/2008	4/7/2009	7/31/2009	7/31/2009	7/27/2009	7/27/2009
		1752H	HT08SOY002-06-32	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1752I	HT08SOY002-06-33	3	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
07	B	1753D	HT08SOY002-07-21	1	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1753E	HT08SOY002-07-22	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1753F	HT08SOY002-07-23	3	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
	C	1753G	HT08SOY002-07-31	1	11/13/2008	4/7/2009	7/31/2009	7/31/2009	7/27/2009	7/27/2009
		1753H	HT08SOY002-07-32	2	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
		1753I	HT08SOY002-07-33	3	11/13/2008	4/7/2009	7/27/2009	7/27/2009	7/27/2009	7/27/2009
08	B	1754D	HT08SOY002-08-21	1	11/13/2008	4/7/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1754E	HT08SOY002-08-22	2	11/13/2008	4/7/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1754F	HT08SOY002-08-23	3	11/13/2008	4/7/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
	C	1754G	HT08SOY002-08-31	1	11/13/2008	4/7/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1754H	HT08SOY002-08-32	2	11/13/2008	4/7/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1754I	HT08SOY002-08-33	3	11/13/2008	4/7/2009	7/31/2009	7/31/2009	7/28/2009	7/28/2009
09	B	1755D	HT08SOY002-09-21	1	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1755E	HT08SOY002-09-22	2	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1755F	HT08SOY002-09-23	3	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
	C	1755G	HT08SOY002-09-31	1	11/13/2008	4/8/2009	7/31/2009	7/31/2009	7/28/2009	7/28/2009
		1755H	HT08SOY002-09-32	2	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1755I	HT08SOY002-09-33	3	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
10	B	1756D	HT08SOY002-10-21	1	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1756E	HT08SOY002-10-22	2	11/13/2008	4/8/2009	7/31/2009	7/31/2009	7/28/2009	7/28/2009
		1756F	HT08SOY002-10-23	3	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
	C	1756G	HT08SOY002-10-31	1	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1756H	HT08SOY002-10-32	2	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009
		1756I	HT08SOY002-10-33	3	11/13/2008	4/8/2009	7/28/2009	7/28/2009	7/28/2009	7/28/2009

Regimen: B = Unsprayed; C = Sprayed with IFT + GLY herbicide

Table A2-4 Validation of 2mEPSPS ELISA for Soybean Grain using Sample BTID 1754A.

ng 2mEPSPS/ mL spiked	Sample I Weight (g/ 4 mL extraction buffer)	Sample II Weight (g/ 4 mL extraction buffer)	Sample III Weight (g/ 4 mL extraction buffer)	Sample IV Weight (g/ 4 mL extraction buffer)	Sample V Weight (g/ 4 mL extraction buffer)
100	0.11	0.11	0.11	0.10	0.11
32	0.11	0.10	0.10	0.11	0.10
16	0.11	0.11	0.11	0.11	0.10
8	0.10	0.10	0.10	0.11	0.10
4	0.10	0.10	0.10	0.10	0.11
2	0.11	0.11	0.11	0.10	0.10
1	0.10	0.11	0.11	0.11	0.10
0.5	0.10	0.11	0.10	0.11	0.10
0	0.10	0.11	0.10	0.10	0.10

ng 2mEPSPS/ mL spiked	Sample I 2mEPSPS 1 (ng/mL)	Sample I 2mEPSPS 2 (ng/mL)	Sample II 2mEPSPS 1 (ng/mL)	Sample II 2mEPSPS 2 (ng/mL)	Sample III 2mEPSPS 1 (ng/mL)	Sample III 2mEPSPS 2 (ng/mL)	Sample IV 2mEPSPS 1 (ng/mL)	Sample IV 2mEPSPS 2 (ng/mL)	Sample V 2mEPSPS 1 (ng/mL)	Sample V 2mEPSPS 2 (ng/mL)	2mEPSPS (ng/mL) Average	2mEPSPS (ng/mL) SD	2mEPSPS (ng/mL) %CV
100	80.3	80.6	82.8	83.7	82.5	77.3	87.0	84.0	85.3	81.9	82.5	2.74	3.32
32	22.3	22.4	22.7	22.8	22.4	23.3	23.1	23.3	23.8	23.5	23.0	0.51	2.21
16	10.4	10.1	10.3	10.5	10.8	10.6	9.91	10.2	10.5	10.4	10.4	0.25	2.45
8	5.27	5.56	5.08	5.26	5.07	5.29	5.02	4.96	4.97	4.88	5.14	0.20	3.98
4	2.56	2.29	2.60	2.62	3.01	2.71	2.45	2.47	2.58	2.62	2.59	0.19	7.23
2	1.07	1.25	1.31	1.27	1.20	1.26	1.13	1.22	1.19	1.20	1.21	0.07	5.74
1	0.47	0.49	0.61	0.75	0.59	0.66	0.56	0.73	0.59	0.71	0.62	0.10	15.7
0.5	0.35	0.76	0.45	0.47	0.44	0.39	0.43	0.44	0.40	0.35	0.45	0.12	26.0
0	0.03	-0.01	0.10	0.22	0.11	0.20	0.09	0.13	0.08	0.11	0.11	0.07	64.2

ng 2mEPSPS/ mL spiked	% 2mEPSPS Recovery 1	% 2mEPSPS Recovery 2	% 2mEPSPS Recovery 3	% 2mEPSPS Recovery 4	% 2mEPSPS Recovery 5	% 2mEPSPS Recovery 6	% 2mEPSPS Recovery 7	% 2mEPSPS Recovery 8	% 2mEPSPS Recovery 9	% 2mEPSPS Recovery 10	% 2mEPSPS Recovery Average	% 2mEPSPS Recovery SD	% 2mEPSPS Recovery %CV
100	80.3	80.6	82.8	83.7	82.5	77.3	87.0	84.0	85.3	81.9	82.5	2.74	3.32
32	69.7	70.0	70.8	71.2	70.0	72.7	72.3	72.9	74.3	73.3	71.7	1.59	2.21
16	64.8	63.1	64.5	65.8	67.3	66.5	61.9	64.0	65.4	64.8	64.8	1.59	2.45
8	65.8	69.5	63.5	65.8	63.3	66.1	62.8	62.0	62.2	61.0	64.2	2.56	3.98
4	64.0	57.3	65.0	65.4	75.3	67.7	61.3	61.7	64.5	65.6	64.8	4.68	7.23
2	53.6	62.7	65.4	63.3	59.9	62.9	56.4	61.0	59.5	60.0	60.4	3.47	5.74
1	47.3	48.6	61.1	75.1	59.0	65.6	56.0	73.2	58.6	70.9	61.5	9.66	15.7
0.5	69.0	151.2	89.6	93.6	88.4	78.8	85.2	88.8	80.2	69.8	89.5	23.2	26.0

Averages, standard deviations and %CV values are calculated with full precision and then rounded to 2 or 3 significant figures.

Table A2-5 Validation of HPPD W336 ELISA for Soybean Grain using Sample BTID 1754A.

ng HPPD W336/ mL spiked	Sample I Weight (g/ 4 mL extraction buffer)	Sample II Weight (g/ 4 mL extraction buffer)	Sample III Weight (g/ 4 mL extraction buffer)	Sample IV Weight (g/ 4 mL extraction buffer)	Sample V Weight (g/ 4 mL extraction buffer)
100	0.11	0.11	0.11	0.10	0.11
32	0.11	0.10	0.10	0.11	0.10
16	0.11	0.11	0.11	0.11	0.10
8	0.10	0.10	0.10	0.11	0.10
4	0.10	0.10	0.10	0.10	0.11
2	0.11	0.11	0.11	0.10	0.10
1	0.10	0.11	0.11	0.11	0.10
0	0.10	0.11	0.10	0.10	0.10

ng HPPD W336/ mL spiked	Sample I HPPD W336 1 (ng/mL)	Sample I HPPD W336 2 (ng/mL)	Sample II HPPD W336 1 (ng/mL)	Sample II HPPD W336 2 (ng/mL)	Sample III HPPD W336 1 (ng/mL)	Sample III HPPD W336 2 (ng/mL)	Sample IV HPPD W336 1 (ng/mL)	Sample IV HPPD W336 2 (ng/mL)	Sample V HPPD W336 1 (ng/mL)	Sample V HPPD W336 2 (ng/mL)	HPPD W336 (ng/mL) Average	HPPD W336 (ng/mL) SD	HPPD W336 (ng/mL) %CV
100	116	124	120	127	124	130	122	122	124	123	123	3.70	3.01
32	32.8	30.6	32.6	30.9	32.4	32.1	30.8	28.6	31.8	31.6	31.4	1.26	4.01
16	18.9	19.5	18.1	18.9	17.4	17.1	16.6	17.0	18.5	17.8	18.0	0.94	5.26
8	8.09	7.23	9.17	8.83	8.65	8.23	8.77	8.13	8.22	8.11	8.34	0.54	6.47
4	3.15	3.14	3.55	3.71	3.65	3.53	3.75	3.28	3.78	3.58	3.51	0.24	6.78
2	0.94	0.97	1.42	1.51	1.53	1.70	1.55	1.56	1.53	1.61	1.43	0.26	18.2
1	0.09	0.13	0.38	0.48	0.44	0.69	0.50	0.61	0.42	0.56	0.43	0.19	44.6
0	-0.67	-0.52	-0.01	-0.15	-0.28	-0.08	-0.33	-0.23	-0.25	0.28	-0.22	0.27	-119

ng HPPD W336/ mL spiked	% HPPD W336 Recovery 1	% HPPD W336 Recovery 2	% HPPD W336 Recovery 3	% HPPD W336 Recovery 4	% HPPD W336 Recovery 5	% HPPD W336 Recovery 6	% HPPD W336 Recovery 7	% HPPD W336 Recovery 8	% HPPD W336 Recovery 9	% HPPD W336 Recovery 10	% HPPD W336 Recovery Average	% HPPD W336 Recovery SD	%HPPD W336 Recovery %CV
100	116	124	120	127	124	130	122	122	124	123	123	3.70	3.01
32	102	95.6	102	96.6	101	100	96.3	89.3	99.5	98.9	98.2	3.94	4.01
16	118	122	113	118	109	107	104	106	116	111	112	5.91	5.26
8	101	90.4	115	110	108	103	110	102	103	101	104	6.75	6.47
4	78.7	78.6	88.9	92.7	91.2	88.3	93.7	82.1	94.5	89.6	87.8	5.95	6.78
2	47.2	48.4	71.0	75.3	76.6	85.2	77.3	77.8	76.7	80.6	71.6	13.1	18.2
1	9.40	12.5	38.3	48.4	44.2	68.6	49.5	61.0	41.7	56.1	43.0	19.2	44.6

Averages, standard deviations and %CV values are calculated with full precision and then rounded to 2 or 3 significant figures.

Table A2-6 Determination of the LOD for 2mEPSPS Protein in Non-transgenic Grain using Sample BTID 1754A

Matrix	ng 2mEPSPS/ mL spiked ^c	Sample I 2mEPS PS 1 (OD)	Sample I 2mEPS PS 2 (OD)	Sample II 2mEPS PS 1 (OD)	Sample II 2mEPS PS 2 (OD)	Sample III 2mEPS PS 1 (OD)	Sample III 2mEPS PS 2 (OD)	Sample IV 2mEPS PS 1 (OD)	Sample IV 2mEPS PS 2 (OD)	Sample V 2mEPS PS 1 (OD)	Sample V 2mEPS PS 2 (OD)	Avg 2mEP SPS (OD)	SD (OD)	3x SD	3x SD + avg ^d	LOD ng/mL ^d	LOD ng/g ^d
Grain ^a	0	0.010	0.003	0.022	0.043	0.024	0.041	0.020	0.028	0.019	0.024	0.023	0.012	0.037	0.060		
	neg. ctrl.	0.013	0.011	0.031	0.000	0.009	0.006	0.028	0.007	0.018	0.027	0.015	0.010	0.031	0.046		
	both											0.019	0.012	0.036	0.055		
Grain ^b	0	-0.009	-0.016	0.003	0.024	0.004	0.022	0.001	0.009	-0.001	0.005	0.004	0.012	0.037	0.041		
	neg. ctrl.	-0.006	-0.008	0.012	-0.019	-0.010	-0.013	0.009	-0.012	-0.001	0.008	-0.004	0.010	0.031	0.027		
	both											0.000	0.012	0.036	0.036	0.258	10.32

Table A2-7 Determination of the LOD for HPPD W336 Protein in Non-transgenic Grain using Sample BTID 1754A

Matrix	ng HPPD W336/ mL spiked ^c	Sample I HPPD W336 1 (OD)	Sample I HPPD W336 2 (OD)	Sample II HPPD W336 1 (OD)	Sample II HPPD W336 2 (OD)	Sample III HPPD W336 1 (OD)	Sample III HPPD W336 2 (OD)	Sample IV HPPD W336 1 (OD)	Sample IV HPPD W336 2 (OD)	Sample V HPPD W336 1 (OD)	Sample V HPPD W336 2 (OD)	Average HPPD W336 (OD)	SD (OD)	3x SD	3x SD + avg ^d	LOD ng/mL ^d	LOD ng/g ^d
Grain ^a	0	-0.035	-0.027	-0.002	-0.009	-0.015	-0.005	-0.018	-0.013	-0.014	0.012	-0.013	0.013	0.039	0.026		
	neg. ctrl.	-0.001	-0.005	-0.019	-0.017	-0.009	-0.008	-0.015	-0.005	0.000	0.000	-0.008	0.007	0.021	0.013		
	both											-0.010	0.010	0.031	0.021		
Grain ^b	0	-0.024	-0.017	0.008	0.001	-0.005	0.005	-0.008	-0.002	-0.004	0.022	-0.002	0.013	0.039	0.036		
	neg. ctrl.	0.009	0.005	-0.009	-0.006	0.001	0.002	-0.005	0.005	0.011	0.010	0.002	0.007	0.021	0.024		
	both											0.000	0.010	0.031	0.031	0.521	20.8

^a OD values were subtracted with the plate background.

^b OD values were subtracted with both the plate background and the average analyte OD value for the measurements calculated from both the 0 ng/mL spike and the negative control.

^c "0" identifies the data for the 0 ng/mL spike. "Neg. ctrl." identifies the data for the negative control on the same plate as the 0 ng/mL spike. "Both" identifies the data obtained by averaging the data from the 0 ng/mL spike and the negative control.

^d The value of 0 ng/g + 3 x SD was entered into the formula for the standard curve on the plate containing the 0 ng/mL spike and the negative control. The result is the LOD in ng/mL which is multiplied by the dilution during extraction (ratio of mL of extraction buffer / grams of matrix extracted) to convert to ng/g.

Table A2-8 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 01

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1747D	B	776	830	857	895	840	50.0	5.95
1747E	B	526	575	643	654	599	60.1	10.0
1747F	B	598	618	781	807	701	108	15.5
					Average	713	124	17.4
1747G	C	615	607	828	966	754	175	23.2
1747H	C	813	829	933	751	831	75.4	9.07
1747I	C	1041	1073	878	792	946	133	14.1
					Average	844	147	17.4

Table A2-9 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 02

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1748D	B	848	911	500	569	707	203	28.7
1748E	B	766	797	756	800	780	22.2	2.85
1748F	B	564	558	425	422	493	79.4	16.1
					Average	660	171	25.9
1748G	C	596	620	381	436	508	118	23.2
1846B	C	326	382	536	587	458	124	27.0
1748I	C	1153	1165	1137	1107	1141	25.3	2.22
					Average	702	337	47.9

Table A2-10 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 03

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1749D	B	811	836	552	552	688	157	22.9
1749E	B	665	691	364	367	522	181	34.7
1749F	B	604	608	412	416	510	111	21.7
					Average	573	162	28.2
1749G	C	616	767	651	747	695	73.2	10.5
1749H	C	527	535	567	554	546	18.2	3.33
1749I	C	737	628	420	461	562	148	26.3
					Average	601	111	18.5

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-11 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 04

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1750D	B	433	444	647	687	553	133	24.1
1750E	B	1080	1088	816	856	960	144	15.0
1750F	B	680	660	904	928	793	143	18.0
					Average	769	216	28.1
1750G	C	1239	1229	1288	1254	1253	25.9	2.07
1750H	C	1166	1140	878	845	1007	169	16.8
1750I	C	893	824	680	654	763	115	15.0
					Average	1008	235	23.3

Table A2-12 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 05

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1751D	B	574	563	665	704	627	69.0	11.0
1751E	B	807	817	546	544	679	154	22.7
1751F	B	507	520	559	512	524	23.7	4.52
					Average	610	111	18.3
1751G	C	1102	1120	677	817	929	218	23.5
1751H	C	1133	1196	790	822	985	209	21.2
1751I	C	1100	1168	857	875	1000	157	15.7
					Average	971	181	18.6

Table A2-13 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 06

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1752D	B	877	948	773	818	854	76.0	8.90
1752E	B	680	727	706	641	689	37.3	5.42
1752F	B	1185	1195	1052	1029	1115	87.1	7.81
					Average	886	194	21.9
1752G	C	613	590	739	720	665	74.8	11.2
1752H	C	1196	1214	941	930	1070	156	14.5
1752I	C	831	804	781	745	790	36.5	4.62
					Average	842	199	23.7

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-14 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 07

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1753D	B	1187	1184	1208	1344	1231	76.1	6.19
1753E	B	1530	1746	1368	1340	1496	186	12.5
1753F	B	1167	1177	956	928	1057	133	12.6
					Average	1261	227	18.0
1753G	C	634	572	639	640	621	33.1	5.33
1753H	C	2041	2296	1542	1854	1933	318	16.4
1753I	C	1037	1055	973	984	1012	39.8	3.93
					Average	1189	598	50.3

Table A2-15 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 08

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1754D	B	1888	1785	1731	1642	1762	103	5.85
1754E	B	2544	2560	3241	3304	2912	417	14.3
1754F	B	2058	2004	2506	2429	2249	255	11.3
					Average	2308	557	24.2
1754G	C	2641	2685	1264	1302	1973	797	40.4
1754H	C	1795	1716	2236	2092	1960	245	12.5
1754I	C	2475	2521	2392	2425	2453	56.6	2.31
					Average	2129	498	23.4

Table A2-16 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 09

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1755D	B	1984	1980	1644	1542	1787	229	12.8
1755E	B	2945	2775	2828	2884	2858	73.5	2.57
1755F	B	2060	2000	2364	2440	2216	218	9.85
					Average	2287	490	21.4
1755G	C	2192	2197	2091	2167	2162	49.0	2.27
1755H	C	932	1056	1188	1144	1080	113	10.5
1755I	C	2680	2585	1964	1856	2271	421	18.6
					Average	1838	607	33.0

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-17 Analytical Results for 2mEPSPS Protein Content in Grain from Trial Site 10

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1756D	B	2910	2681	3332	3290	3053	312	10.2
1756E	B	4859	4804	5470	5788	5230	479	9.16
1756F	B	2375	2307	2488	2550	2430	109	4.50
					Average	3571	1290	36.1
1756G	C	2296	2152	1947	1988	2096	160	7.65
1756H	C	1542	1493	1403	1342	1445	89.8	6.21
1756I	C	1578	1477	1451	1387	1473	79.5	5.40
					Average	1671	331	19.8

Table A2-18 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 01

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1747D	B	973	928	1013	1010	981	39.8	4.06
1747E	B	980	984	1024	1012	1000	21.3	2.13
1747F	B	976	936	1316	1296	1131	203	17.9
					Average	1037	129	12.4
1747G	C	1256	1228	1313	1244	1260	36.9	2.93
1747H	C	1272	1276	749	724	1005	311	30.9
1747I	C	1312	1284	1248	1168	1253	62.4	4.98
					Average	1173	208	17.7

Table A2-19 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 02

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1748D	B	1080	1032	964	960	1009	57.7	5.72
1748E	B	1171	1145	1033	1007	1089	81.1	7.45
1748F	B	1127	1069	833	829	965	156	16.2
					Average	1021	111	10.8
1748G	C	900	888	676	648	778	135	17.3
1846B	C	464	424	698	691	569	146	25.6
1748I	C	1156	1092	1276	1184	1177	76.4	6.49
					Average	841	286	34.0

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-20 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 03

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1749D	B	961	980	772	794	877	109	12.4
1749E	B	860	904	532	542	710	200	28.2
1749F	B	1220	1196	1004	964	1096	131	11.9
					Average	894	215	24.0
1749G	C	975	902	1000	1004	970	47.3	4.88
1749H	C	916	876	932	880	901	27.4	3.04
1749I	C	1052	1000	932	892	969	71.1	7.33
					Average	947	57.7	6.10

Table A2-21 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 04

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1750D	B	767	756	928	928	845	96.0	11.4
1750E	B	980	972	924	924	950	30.4	3.20
1750F	B	756	714	923	889	820	101	12.3
					Average	872	94.7	10.9
1750G	C	693	640	769	723	707	54.1	7.66
1750H	C	1190	1027	893	831	985	159	16.2
1750I	C	936	840	1004	956	934	68.9	7.37
					Average	875	158	18.1

Table A2-22 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 05

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1751D	B	592	580	616	628	604	21.9	3.63
1751E	B	656	664	480	455	564	112	19.8
1751F	B	760	756	512	492	630	148	23.5
					Average	599	102	17.0
1751G	C	792	784	612	608	699	103	14.7
1751H	C	633	600	548	516	574	52.2	9.09
1751I	C	868	804	680	644	749	105	14.0
					Average	674	112	16.6

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-23 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 06

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1752D	B	888	920	812	812	858	54.7	6.38
1752E	B	669	680	578	564	623	60.3	9.68
1752F	B	938	931	924	960	938	15.6	1.66
					Average	806	146	18.2
1752G	C	796	756	584	568	676	117	17.3
1752H	C	1072	972	880	768	923	130	14.1
1752I	C	724	676	669	647	679	32.3	4.76
					Average	759	152	20.1

Table A2-24 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 07

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1753D	B	596	612	705	691	651	55.1	8.46
1753E	B	888	920	744	768	830	87.0	10.5
1753F	B	720	767	632	648	692	63.2	9.14
					Average	724	102	14.1
1753G	C	888	872	548	524	708	199	28.1
1753H	C	740	716	676	644	694	42.5	6.13
1753I	C	708	652	676	695	683	24.2	3.55
					Average	695	108	15.5

Table A2-25 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 08

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1754D	B	592	600	572	584	587	11.9	2.03
1754E	B	588	596	640	622	611	23.9	3.91
1754F	B	604	622	728	764	679	78.7	11.6
					Average	626	59.6	9.52
1754G	C	556	567	411	411	486	87.2	17.9
1754H	C	585	542	584	544	564	24.2	4.29
1754I	C	616	580	771	698	666	85.5	12.8
					Average	572	101	17.6

Regimen B=Unsprayed

Regimen C=Sprayed with one application IFT + GLY herbicide

Table A2-26 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 09

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1755D	B	880	848	636	600	741	143	19.3
1755E	B	1076	1051	1140	1064	1083	39.5	3.65
1755F	B	832	856	1040	1032	940	111	11.8
					Average	921	176	19.1
1755G	C	1020	1008	1015	989	1008	13.5	1.34
1755H	C	588	612	632	636	617	22.0	3.57
1755I	C	924	876	780	848	857	60.1	7.01
					Average:	827	172	20.7

Table A2-27 Analytical Results for HPPD W336 Protein Content in Grain from Trial Site 10

Sample ID	Regimen	Sample I a (ng/g)	Sample I b (ng/g)	Sample II a (ng/g)	Sample II b (ng/g)	Average (ng/g)	SD (ng/g)	%CV
1756D	B	1056	1024	1098	1098	1069	36.0	3.37
1756E	B	1036	993	1189	1102	1080	85.4	7.91
1756F	B	731	724	724	696	719	15.5	2.15
					Average	956	182	19.0
1756G	C	553	545	651	636	596	55.0	9.22
1756H	C	684	662	655	644	661	16.9	2.56
1756I	C	748	724	676	698	712	31.1	4.37
					Average	656	59.9	9.13