In vitro Digestibility of *p*-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein under Simulated Mammalian Gastric Conditions

Data Requirement: Not applicable

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Study Completion Date: August 24, 2010

Syngenta Study No.: TKRS0000157

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Company: *Syngenta Seeds, Inc.*

Company Representative:

[redacted]

20 Aug 2010

Date

Senior Regulatory Affairs Manager

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STATEMENT CONCERNING GOOD LABORATORY PRACTICES STANDARDS

With the exceptions noted below, this study was conducted in compliance with the relevant provisions of Good Laboratory Practices Standards (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act.

- The SeeBlue® Plus2 molecular weight standard was characterized by the manufacturer, Invitrogen, Inc. (Carlsbad, CA, USA), prior to use in this study. This characterization was not conducted under Good Laboratory Practices Standards.

Study Director:

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QUALITY ASSURANCE STATEMENT

Study Title: *In vitro* Digestibility of p-Hydroxyphenylpyruvate Dioxygenase (AvHPPD-03) Protein under Simulated Mammalian Gastric Conditions

Study Director: [redacted]

Study Number: TKRS0000157

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Audit Protocol	October 1, 2009	October 1, 2009
Inspect Analytical	October 23, 2009	October 23, 2009
Final Report Audit (1st audit)	July 21, 2010	July 21, 2010
Final Report Audit (2nd audit)	August 2 – 3, 2010	August 3, 2010

Prepared by: [redacted] Date: Aug. 4, 2010

Staff Quality Assurance Auditor
Quality Assurance Unit
Syngenta Crop Protection, Inc.

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LIST OF ACRONYMS AND ABBREVIATIONS

AvHPPD-03	*p*-hydroxyphenylpyruvate dioxygenase from *Avena sativa*
Bis-Tris	(Bis(2-hydroxyethyl)-amino-tris(hydroxymethyl)-methane)
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
HPPD	*p*-hydroxyphenylpyruvate dioxygenase
LOD	limit of detection
MES	2-(*N*-morpholino) ethanesulfonic acid
mM	millimolar
ng	nanogram
PAGE	polyacrylamide gel electrophoresis
PVDF	polyvinylidene difluoride
SDS	sodium dodecylsulfate
SGF	simulated mammalian gastric fluid
US EPA	United States Environmental Protection Agency
µg	microgram
®	registered trademark

SUMMARY

The susceptibility of *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) to proteolytic degradation in simulated mammalian gastric fluid (SGF) containing pepsin was evaluated *via* sodium dodecylsulfate polyacrylamide gel electrophoresis and Western blot analyses.

The AvHPPD-03 protein degraded rapidly upon exposure to SGF. No intact AvHPPD-03 (molecular weight approximately 47.0 kDa) or AvHPPD-03 derived fragments were detected following its incubation in SGF for one minute.

The results of this study support the conclusion that AvHPPD-03 will be readily digested under typical mammalian gastric conditions.

INTRODUCTION

The purpose of this study is to assess the *in vitro* digestibility of *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) in simulated mammalian gastric fluid (SGF). Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses were used to evaluate the *in vitro* digestibility of AvHPPD-03 in SGF over a 60 minute time course at 37ºC.

The AvHPPD-03 protein was prepared from a recombinant *Escherichia coli* strain expressing the novel gene, *avhppd-03* derived from oat (*Avena sativa*). The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor of plastoquinone and vitamin E biosynthesis. Expression of *avhppd-03* in plants confers a mesotrione-tolerance phenotype.

MATERIALS AND METHODS

Microbially Produced AvHPPD-03

Microbially produced AvHPPD-03 test substance was prepared from an *Escherichia coli* (*E. coli*) expression system. The *avhppd-03* gene was introduced into a pET24a vector and transformed into *E. coli* strain BL21 (DE3) cells.

In August 2009, AvHPPD-03 was prepared from *E. coli* cell paste by Syngenta Protein Science (Jealott's Hill International Research Centre, Bracknell, UK). Briefly, *E. coli* cells were ruptured and the cell debris was removed by centrifugation. The AvHPPD-03 protein was further purified using anion exchange chromatography, hydrophobic interaction chromatography and gel filtration chromatography. The purified protein was pooled, concentrated, aliquoted, and lyophilized. The resulting lyophilized powder was designated test substance AVHPPD-03-0209. The test substance was shipped on dry ice to Syngenta Biotechnology, Inc. (Research Triangle Park, NC, USA), where it was stored at -20°C ±8°C.

This test substance was the source of AvHPPD-03 in this study. AVHPPD-03-0209 was characterized in detail and was determined to contain 72.2% AvHPPD-03 by weight; the molecular weight of AvHPPD-03 was consistent with the predicted molecular weight of 47.0 kDa (Winslow 2009). For use in this study, AvHPPD-03-0209 was solubilized in purified water.

In vitro Digestibility of AvHPPD-03 under Simulated Mammalian Gastric Conditions

The SGF digestibility assay was performed at 37°C ±2°C over a 60 minute time course with samples taken at 0, 1, 2, 5, 10, 30, and 60 minutes.

The SGF (USP 2000) was prepared the day of use and consisted of 2 mg/ml sodium chloride (pH 1.2), and pepsin at approximately 2,600 units/ml (Sigma-Aldrich Cat. No. P6887).

The digestion reaction was initiated by the addition of AvHPPD-03 to SGF at a ratio of 1 µg AvHPPD-03 per 10 pepsin activity units (Thomas *et al.* 2004); the reaction mixture was incubated at 37°C ±2°C. At each time point, an aliquot of the reaction mixture was removed and mixed with stop solution (described below) to terminate the reaction. The samples were then incubated for 10 minutes at 95°C ±5°C in preparation for SDS-PAGE analysis.

The time zero (T0) sample was prepared by adding an aliquot of AvHPPD-03 to a mixture of SGF and stop solution. It was then prepared for SDS-PAGE analysis as described above. Mixing the SGF with the stop solution ensures the inactivation of pepsin before AvHPPD-03 is added for an accurate time zero. This time zero sample serves as an undigested control to which all samples are visually compared, allowing the digestion of AvHPPD-03 in SGF to be assessed over the time course.

The stop solution was a mixture of 200 mM sodium bicarbonate (pH 11.0) and lithium dodecyl sulfate sample buffer. Upon the addition of the stop solution, pepsin was inactivated by the resulting pH shift which terminated the reaction. All samples were stored at -20°C ±8°C until SDS-PAGE and Western blot analyses were conducted.

Table 1 details the composition of the SGF *in vitro* digestibility assay samples.

Assay Control Samples

Two controls were utilized in this study, an AvHPPD-03 control and an SGF control.

The AvHPPD-03 control contained AvHPPD-03 and SGF without pepsin. This control examined the potential hydrolysis of AvHPPD-03 in SGF without pepsin over the 60 minute time course.

The SGF control contained SGF and purified water, the solvent used to prepare AvHPPD-03. This control was examined to evaluate the potential for self hydrolysis of pepsin over the 60 minute time course.

Both controls had the same concentration of AvHPPD-03 or pepsin as initially contained in the *in vitro* digestibility assay samples. Both AvHPPD-03 and SGF controls were incubated at 37°C ±2°C and sampled at 0 and 60 minutes. Each control was combined with the stop solution and prepared for SDS-PAGE analysis in the same manner as described above for the *in vitro* digestibility assay samples. All assay control samples were stored at -20°C ±8°C until SDS-PAGE and Western blot analyses were conducted.

Table 1 details the composition of the assay control samples.

Table 1. SGF *in vitro* digestibility time course and control samples

Name	Composition	Time points examined	Purpose
SGF *in vitro* digestibility assay samples	AvHPPD-03, SGF	T0 (undigested control), 1, 2, 5, 10, 30, and 60 minutes	Examined the *in vitro* digestibility of AvHPPD-03 in SGF
AvHPPD-03 control	AvHPPD-03, SGF without pepsin	T0 and 60 minutes	Examined the potential hydrolysis of AvHPPD-03 in SGF without pepsin
SGF control	purified water[1], SGF	T0 and 60 minutes	Examined the potential for self-hydrolysis of pepsin over the 60 minute time course

[1]Purified water was the solvent in which AvHPPD-03 was solubilized
All samples and controls were analyzed *via* SDS-PAGE and Western blot analyses

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis Analysis

Equivalent volumes of each *in vitro* digestibility assay sample were subjected to SDS-PAGE under reducing conditions using a 4-12% Bis-Tris gel and 2-(*N*-morpholino) ethanesulfonic acid (MES) running buffer. Based on the concentration of AvHPPD-03 and the amount of pepsin at the initiation of the digestion reaction, these volumes were equivalent to 1 µg AvHPPD-03 and 10 Units of pepsin. Volumes of the control samples, containing either 1 µg AvHPPD-03 or 10 units of pepsin, were subjected to SDS-PAGE (described above). The molecular weight standard was SeeBlue® Plus2 pre-stained standard. The gel was stained with Coomassie® blue and examined for the presence of bands consistent with the molecular weight of intact AvHPPD-03 (approximately 47.0 kDa).

The limit of detection (LOD) is defined as the lowest amount of analyte in a sample that can be detected. The LOD for SDS-PAGE was determined by subjecting serial dilutions of AvHPPD-03 to SDS-PAGE. Amounts tested were 0.063, 0.031, 0.016, 0.0078 and 0.0039 µg of AvHPPD-03. The gel was stained with a Coomassie® stain and the lowest amount of AvHPPD-03 visible on the gel was designated the LOD of AvHPPD-03 for SDS-PAGE.

Western Blot Analysis

Equivalent volumes of each *in vitro* digestibility assay sample were subjected to SDS-PAGE. Based on the concentration of AvHPPD-03 and amount of pepsin at the initiation of the digestion reaction, these volumes were equivalent to 5 ng AvHPPD-03 and 0.05

units of pepsin (equivalent to a ratio of 1 µg AvHPPD-03 to 10 units of pepsin). Volumes of the control samples, containing either 5 ng AvHPPD-03 or 0.05 units of pepsin, were also subjected to SDS-PAGE. The molecular weight standard was SeeBlue® Plus 2 pre-stained standard. Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane *via* electroblotting. The membrane was probed with a polyclonal rabbit antibody capable of detecting AvHPPD-03. An alkaline phosphatase conjugated donkey anti-rabbit antibody was used to bind to the primary antibody. The protein was visualized by developing the blot with an alkaline phosphatase substrate solution. The Western blot was examined for the presence of bands consistent with the molecular weight of intact AvHPPD-03 (approximately 47.0 kDa) and AvHPPD-03 derived fragments.

The LOD for Western blot analysis was determined by subjecting serial dilutions of AvHPPD-03 to SDS-PAGE. Amounts tested were 0.16, 0.078, 0.039, 0.02 and 0.0098 ng AvHPPD-03. The protein was transferred to a PVDF membrane and then probed with the same antibodies used to monitor the SGF assay. The lowest amount of AvHPPD-03 visible on the membrane was designated the LOD of AvHPPD-03 for the Western blot analysis.

Statistical Analysis

No statistical analysis was required for any parameter evaluated in this study.

RESULTS AND DISCUSSION

Two methods (SDS-PAGE and Western blot) were used to analyze all samples and controls of the AvHPPD-03 *in vitro* digestibility experiment. The SDS-PAGE analysis, using a non-specific Coomassie® protein stain, allows for visualization of all proteins present in a sample. The Western blot method allows for the specific analysis of the AvHPPD-03 protein within the *in vitro* digestibilty assay. Figures 1 and 2 show the results of the SDS-PAGE and Western blot analyses, respectively.

Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis Analysis

The SDS-PAGE analysis (Figure 1) results suggest that AvHPPD-03 is readily digested after incubation in SGF for one minute. The band corresponding to AvHPPD-03 at time zero (Lane 7) was no longer visible after incubation in SGF for one minute (Lane 8). No additional bands were observed at later time points (Lanes 9 through 13), indicating there are no breakdown fragments of AvHPPD-03 after incubation in SGF for one minute.

The approximately 35 kDa protein band, present in the SGF control at 0 and 60 minutes (Lanes 2 and 3), corresponds to the molecular weight of pepsin (MW 34.6 kDa[1]). The 34.6 kDa pepsin band was also visible in the *in vitro* digestibility assay samples (Lanes 8 through 13). The intensity of the pepsin band in the SGF control at 0 and 60 minutes (Lanes 2 and 3) are very similar in intensity; therefore, there was no significant degradation of pepsin observed over the 60 minute time course.

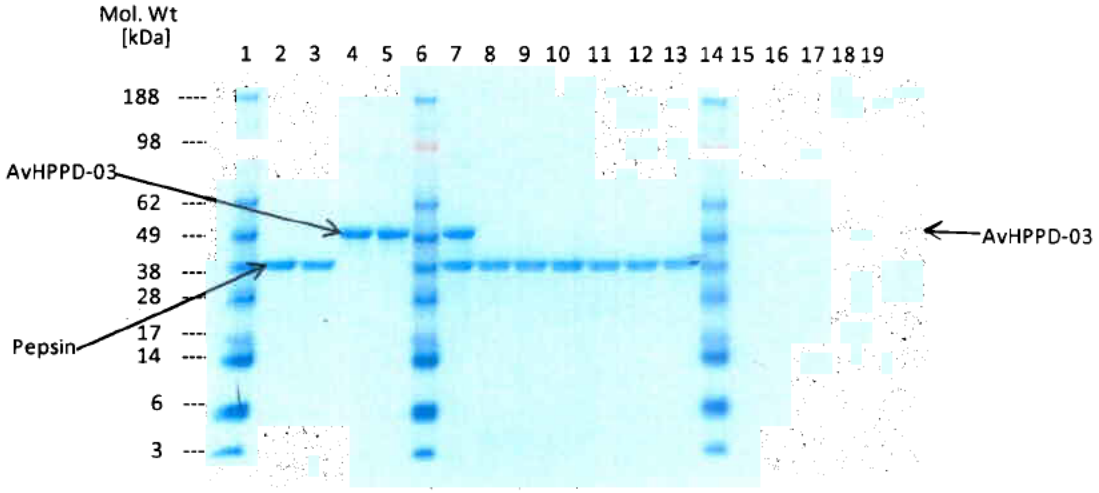
Similar band intensities were visualized between the time zero *in vitro* digestibility assay sample (Lane 7) and the AvHPPD-03 time zero control (Lane 4), confirming that equal amounts were applied to the SDS-PAGE.

The AvHPPD-03 protein incubated in SGF without pepsin (AvHPPD-03 control) showed no significant degradation after 60 minutes (Lanes 4 and 5), indicating that the hydrolysis of AvHPPD-03 seen for the SGF samples (Lanes 8 through 13) can be attributed to the hydrolytic activity of pepsin.

The lowest amount of AvHPPD-03 visible on the gel (Lanes 15 through 19) was 0.0078 µg (Lane 18). Therefore, the LOD of AvHPPD-03 for SDS-PAGE used in this study was determined to be 0.0078 µg.

[1] The 34.6 kDa pepsin bands showed slightly lower mobility and, therefore an apparent higher molecular weight, when compared to the molecular weight standards on the gel (Figure 1). The difference between the expected and observed molecular weights on the gel can be explained by the limitations of SDS-PAGE for accurate determination of molecular weight. Dube and Flynn (1988) have reviewed the reliability of SDS-PAGE for molecular weight determinations and concluded that the apparent molecular weight of a protein by this method is typically within 10% of its true molecular weight. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi *et al.*, 2003).

Figure 1. SDS-PAGE analysis of AvHPPD-03 following digestion in SGF



Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 60 minutes
Lane 4: AvHPPD-03 control (AvHPPD-03 in SGF without pepsin) – time zero
Lane 5: AvHPPD-03 control (AvHPPD-03 in SGF without pepsin) – 60 minutes
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero
Lane 8: *in vitro* digestibility assay - 1 minute
Lane 9: *in vitro* digestibility assay - 2 minutes
Lane 10: *in vitro* digestibility assay - 5 minutes
Lane 11: *in vitro* digestibility assay - 10 minutes
Lane 12: *in vitro* digestibility assay - 30 minutes
Lane 13: *in vitro* digestibility assay - 60 minutes
Lane 14: molecular weight standard
Lane 15: 0.063 µg AvHPPD-03 for LOD determination
Lane 16: 0.031 µg AvHPPD-03 for LOD determination
Lane 17: 0.016 µg AvHPPD-03 for LOD determination
Lane 18: 0.0078 µg AvHPPD-03 for LOD determination
Lane 19: 0.0039 µg AvHPPD-03 for LOD determination

The predicted molecular weight of AvHPPD-03 is 47.0 kDa.[2]

Western Blot Analysis

The Western blot analysis (Figure 2) results confirm that AvHPPD-03 is readily digested in SGF. The band corresponding to intact AvHPPD-03 at time zero (Lane 7) is no longer visible after incubation in SGF for one minute (Lane 8). This result strongly supports the conclusion that no intact AvHPPD-03 was present after one minute.

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[2] The 47.0 kDa AvHPPD-03 protein band showed slightly lower mobility and, therefore an apparent higher molecular weight, when compared to the molecular weight standards on the gel and Western blot (Figure 1 and Figure 2). The difference between the expected and observed molecular weights on the gel can be explained by the limitations of SDS-PAGE for accurate determination of molecular weight. Dube and Flynn (1988) have reviewed the reliability of SDS-PAGE for molecular weight determinations and concluded that the apparent molecular weight of a protein by this method is typically within 10% of its true molecular weight. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi *et al.*, 2003).

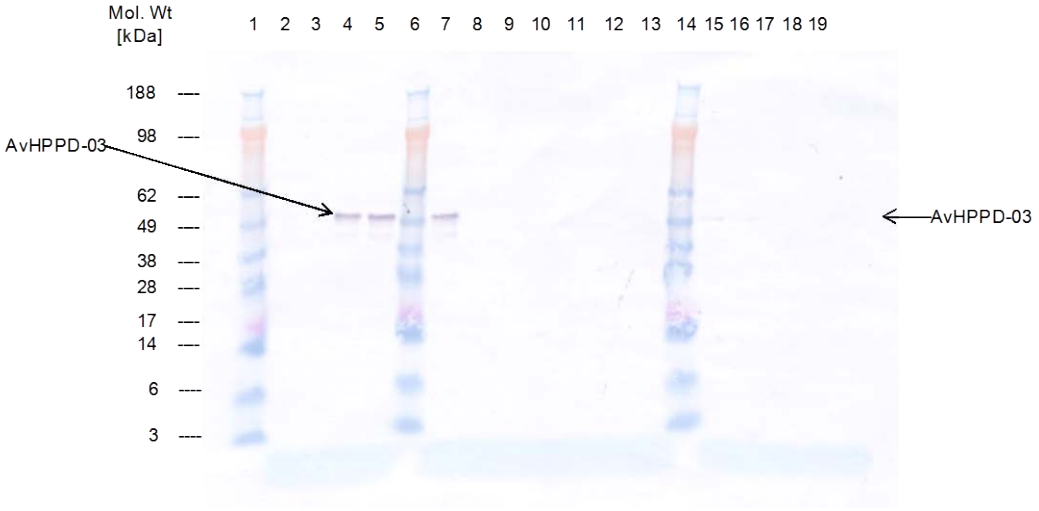
Faint, diffuse bands with approximate molecular weights of 42 kDa and 45 kDa were visible in the T0 sample (Lane 7). These bands cross reacted with the antibody capable of detecting AvHPPD-03 and were also present in the AvHPPD-03 control (Lanes 4 and 5). This suggests the bands most likely correspond to minor AvHPPD-03 degradation products derived from the sample and are not related to pepsin digestion. These bands, along with the fully intact AvHPPD-03, were no longer detected following exposure to SGF for one minute.

Similar band intensities were visualized between the time zero *in vitro* digestibility assay sample (Lane 7) and the AvHPPD-03 time zero control (Lane 4), confirming that equal amounts were applied to the Western blot.

The AvHPPD-03 protein incubated in SGF without pepsin (AvHPPD-03 control) showed no significant degradation over the 60 minutes (Lane 5) indicating that the hydrolysis of AvHPPD-03 seen for the SGF samples (Lanes 8 through 13) can be attributed to the hydrolytic activity of pepsin.

The lowest amount of AvHPPD-03 visible on the Western blot (Lanes 15 through 19) was 0.078 ng (Lane 16). Therefore, the LOD of AvHPPD-03 for the Western blot used in this study was determined to be 0.078 ng.

Figure 2. Western blot analysis of AvHPPD-03 following digestion in SGF



Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 60 minutes
Lane 4: AvHPPD-03 control (AvHPPD-03 in SGF without pepsin) – time zero
Lane 5: AvHPPD-03 control (AvHPPD-03 in SGF without pepsin) – 60 minutes
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero
Lane 8: *in vitro* digestibility assay - 1 minute
Lane 9: *in vitro* digestibility assay - 2 minutes
Lane 10: *in vitro* digestibility assay - 5 minutes
Lane 11: *in vitro* digestibility assay - 10 minutes
Lane 12: *in vitro* digestibility assay - 30 minutes
Lane 13: *in vitro* digestibility assay - 60 minutes
Lane 14: molecular weight standard
Lane 15: 0.16 ng AvHPPD-03 for LOD determination
Lane 16: 0.078 ng AvHPPD-03 for LOD determination
Lane 17: 0.039 ng AvHPPD-03 for LOD determination
Lane 18: 0.020 ng AvHPPD-03 for LOD determination
Lane 19: 0.0098 ng AvHPPD-03 for LOD determination

The predicted molecular weight of AvHPPD-03 is 47.0 kDa.[3]

Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

[3]The 47.0 kDa AvHPPD-03 protein band showed slightly lower mobility and, therefore an apparent higher molecular weight, when compared to the molecular weight standards on the gel and Western blot (Figure 1 and Figure 2). The difference between the expected and observed molecular weights on the gel can be explained by the limitations of SDS-PAGE for accurate determination of molecular weight. Dube and Flynn (1988) have reviewed the reliability of SDS-PAGE for molecular weight determinations and concluded that the apparent molecular weight of a protein by this method is typically within 10% of its true molecular weight. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi *et al*., 2003).

CONCLUSIONS

The AvHPPD-03 protein degraded rapidly upon exposure to SGF. No intact AvHPPD-03 or AvHPPD-03 derived fragments were detected following its incubation in SGF for 1 minute.

The results of this study support the conclusion that AvHPPD-03 will be readily digested under typical mammalian gastric conditions.

RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CONTRIBUTING SCIENTISTS

The analytical work reported herein was conducted by [redacted] at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CRITICAL DATES

Study initiation date:	October 15, 2009
Experimental start date:	October 23, 2009
Experimental end date:	November 17, 2009

REFERENCES

Dube S, Flynn E. 1988. Estimating protein molecular weights using SDS-PAGE. *Focus* 20:24-25.

Sadeghi M, Hajivandi M, Bogoev R, Amshey J. 2003. Molecular weight estimation of proteins by gel electrophoresis revisited. *Focus* 25:35-39.

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu TJ, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herout C, Halsappe M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, van Ree R, Woolhiser M, Zawodny J. 2004. A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regul Toxicol Pharmacol* 39:87–89.

US EPA. 1989. Good Laboratory Practices Standards. 40 CFR Part 160.

USP. 2000. *The United States Pharmacopeia, 24th rev. The National Formulary, 19th ed.* Rockville, MD: United States Pharmacopoeial Convention, Inc. p. 2235.

Winslow S. 2009. *Characterization of Microbially Produced Test Substance Containing p-Hydroxyphenylpyruvate Dioxygenase Protein (AvHPPD-03) and Certificate of Analysis*. Report No. SSB-041-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology.