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STUDY TITLE

Endogenous Allergen Analysis of DAS-44406-6 Soybean

DATA REQUIREMENTS

Assessment of allergenicity of whole GM plant or crop

AUTHOR(S)

N.V.J. Harpham, E. Ma, and N.J. Stagg

STUDY COMPLETED ON

10 – June – 2011

PERFORMING LABORATORY

Regulatory Sciences and Government Affairs—Indianapolis Lab  
Dow AgroSciences LLC  
9330 Zionsville Road  
Indianapolis, Indiana 46268-1054

LABORATORY STUDY ID

110512

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## Endogenous Allergen Analysis of DAS-444Ø6-6 Soybean

SUMMARY

As soybean has a history of causing food allergy, a sera screening study was conducted to determine if the genetic modification used to generate DAS-444Ø6-6 soybean altered the endogenous allergen content of soybean. DAS-444Ø6-6 soybean was compared to its non-transgenic counterpart (Maverick) by one dimensional (1D) IgE immunoblot (qualitative analysis) and ELISA inhibition (a quantitative assessment). The results of this study demonstrate that DAS-444Ø6-6 soybean and non-GM control (Maverick) are similar in endogenous allergen profiles. Therefore, the genetic modification used to generate DAS-444Ø6-6 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

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AUTHOR(S)

N.V.J. Harpham  
317-337-4940  
[njharpham@dow.com]

E. Ma  
317-337-3229  
[hma2@dow.com]

N.J. Stagg  
317-337-4548  
[njstagg@dow.com]

STUDY COMPLETED ON

10 – June – 2011

PERFORMING LABORATORY

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Dow AgroSciences LLC  
9330 Zionsville Road  
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Compound: DAS-44406-6

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Company: Dow AgroSciences LLC

Company Agent: M. Krieger, Ph.D.

Title: Regulatory Manager

Signature: 

Date: 9 June 2014

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## STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Endogenous Allergen Analysis of DAS-44406-6 Soybean

Study Initiation Date: 1st March 2011

This report does not meet the definition of GLP study by EPA FIFRA Good Laboratory Practice Standards.

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

Organisation for Economic Co-Operation and Development  
ENV/MC/CHEM(98)17, Paris January 26, 1998

  
\_\_\_\_\_  
M. Krieger, Ph.D.  
Sponsor  
Dow AgroSciences LLC  
Date 9 June 2011

  
\_\_\_\_\_  
M. Krieger, Ph.D.  
Submitter  
Dow AgroSciences LLC  
Date 9 June 2011

  
\_\_\_\_\_  
N.V.J. Harpham, Ph.D.  
Author  
Dow AgroSciences LLC  
Study Completion Date 10-June-2011

## QUALITY ASSURANCE STATEMENT

Compound: DAS-44406-6

Title: Endogenous Allergen Analysis of DAS-44406-6 Soybean

Study Initiation Date: 1-March-2011

Study Completion Date: 10 – June – 2011

## **NON-GLP STUDY**

SIGNATURE PAGE

  
\_\_\_\_\_  
N.V.J. Harpham  
Author  
Dow AgroSciences LLC

7-June-2011  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
N.J. Stagg  
Co-author  
Dow AgroSciences LLC

7-jun-2011  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
E. Ma  
Co-author  
Dow AgroSciences LLC


7-Jun-2011  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
G. Shan  
Science Leader  
Dow AgroSciences LLC

7 Jun 2011  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
B.W. Schafer  
Peer Reviewer  
Dow AgroSciences LLC

07-JUN-2011  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
D. Juerg  
NA Leader, Human Health Assessment  
Dow AgroSciences LLC

08 June 2011  
\_\_\_\_\_  
Date

\_\_\_\_\_

## STUDY PERSONNEL

Title: Endogenous Allergen Analysis of DAS-44406-6 Soybean

Study Director: N.V.J Harpham

Analyst: N.V.J. Harpham, E. Ma; S. Embrey



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## Endogenous Allergen Analysis of DAS-44406-6 Soybean

### **ABSTRACT**

As soybean has a history of causing food allergy, a sera screening study was conducted to determine if the genetic modification used to generate DAS-44406-6 soybean altered the endogenous allergen content of soybean. DAS-44406-6 soybean was compared to its non-transgenic counterpart (Maverick) by one dimensional (1D) IgE immunoblot (qualitative analysis) and ELISA inhibition (a quantitative assessment). The results of this study demonstrate that DAS-44406-6 soybean and non-GM control (Maverick) are similar in endogenous allergen profiles. Therefore, the genetic modification used to generate DAS-44406-6 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

## INTRODUCTION

Soybean is listed as one of the eight most common allergenic foods in the United States of America and one of the 12 most common allergic foods in Europe (1, 2). Therefore, a study was conducted to determine if the genetic modification used to generate DAS-444Ø6-6 soybean altered the endogenous allergen content compared to the non-transgenic control (Maverick).

IgE binding to extracts of DAS-444Ø6-6 soybean and its non-transgenic control (Maverick) were evaluated with one dimensional (1D) IgE immunoblot (qualitative analysis) and ELISA inhibition (quantitative analysis) using sera from 10 clinically-reactive soy allergic patients.

Human clinical sera were obtained from Plasma Lab International, Everett, WA.

## SDS-PAGE IMMUNOBLOT ANALYSIS

To extract the soluble protein from the both Maverick and DAS-444Ø6-6 soybean seeds, the soybean seed powders were removed from -80 °C and approximately 12.5 g was weighed into appropriate containers and 125 mL (1:10 w/v) of extraction buffer (50mM Tris-HCl, 100mM NaCl, 5mM EDTA, pH 8.5) was added to each sample. The samples were covered with foil and the samples were incubated overnight at 4 °C. Samples were removed from 4 °C and homogenized at room temperature for 3 minutes using a Cuisinart Smart Stick homogenizer. Samples were then centrifuged at 3,000 x g for 30 minutes and the resulting supernatants were vacuum filtered through P8 grade filter paper. Extracts were further clarified by centrifugation at 3,000 x g for 30 minutes at 4 °C. The supernatants were then filtered through a 0.22 µm sterile filter and collected into sterile 50mL centrifuge tubes. The resulting sample was aliquoted into 1-mL sub-aliquots and stored in a -80 °C freezer. All samples were mixed with Laemmli buffer and heated at 95 °C for 5 min and then subjected to SDS-PAGE with Coomassie blue staining to evaluate protein content of the two seed lots. Gel transfer of the proteins to a nitrocellulose membrane was performed with blots replicating the SDS-PAGE. Transfer was confirmed with the use of pre-stained molecular weight markers. The blotted membranes were blocked with 5% non-fat milk in PBST for at least 1 hour at room temperature followed by overnight incubation at

4 °C in serum (1:20 dilution) from a pool of 10 soy-allergic patients. The blots were washed with PBST to remove unbound IgE and then incubated in biotinylated goat anti-human IgE antibody for 1 hour at room temperature with continuous agitation. Additional washing with PBST was carried out and then the blots were incubated with NeutrAvidin-HRP (horse radish peroxidase) conjugate for 30 minutes at room temperature. GE Healthcare ECL Plus chemiluminescent substrate was used for development and visualization of the immunoreactive protein bands. The membranes were covered with ECL Plus reagent for 5 minutes, excess solution removed and exposed to Thermo Scientific CLX-Posure film in a darkroom and developed.

## Results

The protein profiles between DAS-44406-6 and the non-transgenic soybean line, Maverick were compared using SDS-PAGE analysis with Coomassie blue staining, which did not reveal any differences in protein banding patterns between the two soybean extracts. The IgE binding profiles of DAS-44406-6 and Maverick were compared in the one-dimensional immunoblot using soy-allergic sera and also demonstrated no differences (Figure 1).

## **ELISA INHIBITION**

ELISA inhibition of IgE binding from a pooled soybean-allergic serum sample was conducted for DAS-44406-6 and control (Maverick) soybean extracts. Extracts from DAS-44406-6 and Maverick (at various concentrations: 0.0075 to 7500 µg/ml of total soluble protein) were pre-incubated with the pooled serum and transferred to 96-well plates previously coated with non-transgenic control (Maverick) extracts at 25 µg/mL (100 µL/well). After a washing step with PBST, biotinylated goat-anti-human IgE antibody and NeutraAvidin-HRP conjugate were sequentially incubated on the plates with a PBST washing step after each incubation. The plates were lastly incubated with peroxidase substrate TMB and the reaction was stopped with 1 N HCl. A microtiter plate reader was used to measure the absorbance in the wells at 450 nm with 650nm background subtraction.

The results of the ELISA inhibition experiments were plotted and analyzed using GraphPad Prism 4 (GraphPad Software Inc, La Jolla, CA). Data were analyzed using a non-linear regression curve fit for a sigmoidal dose-response with a variable slope. This approach uses the following equation, which is identical to the four parameter logistic equation:  $Y = \text{Bottom} + ((\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) * \text{HillSlope}}))$ . X is the logarithm of the protein concentration, and Y is the percent inhibition. Constraints were applied to set the Bottom  $\geq$  0% and the Top  $\leq$  100%. The EC<sub>50</sub> value from this analysis represents the protein concentration at which the Y value of the curve (% Inhibition) is halfway between the Top and Bottom plateaus of the curve. The EC<sub>50</sub> values and their associated 95% confidence intervals are plotted for the Maverick soybean and DAS-44406-6 extracts.

## Results

The ELISA inhibition data with the pooled soy-allergic serum showed the same IgE binding response between the non-transgenic Maverick soybean and DAS-44406-6 soybean extracts against 2.5 µg/well of immobilized Maverick extracts on the plate (Figure 2). Furthermore, the associated EC<sub>50</sub> values and 95% confidence intervals for Maverick and DAS-44406-6 were similar (Figure 3).

## **CONCLUSION**

The immunoblot and ELISA inhibition data demonstrate that the genetic modification used to generate DAS-44406-6 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

## **ARCHIVING STATEMENT**

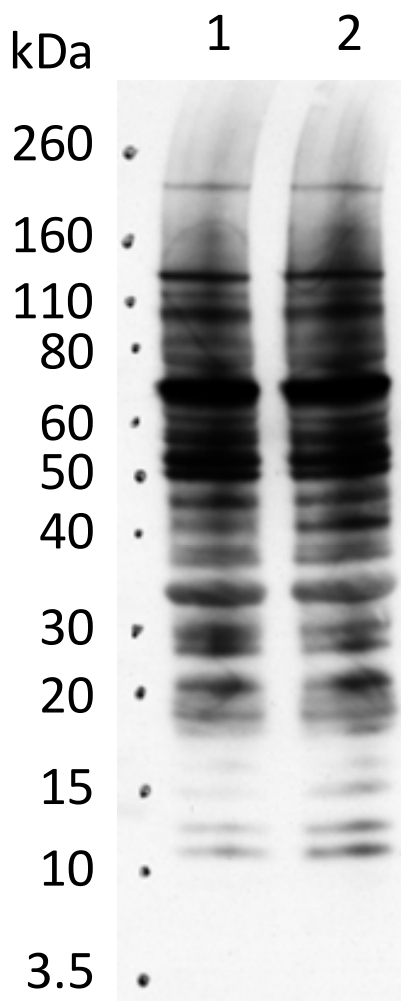
The protocol, raw data, and the original version of the final report will all be filed in the Dow Agrosciences LLC archives at 9330 Zionsville road Indianapolis, IN 46268-1054

## REFERENCES

(1) European Food Safety Authority (EFSA). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to a notification from FEDIOL and IMACE on fully refined soybean oil and fat pursuant to Article 6, paragraph 11 of Directive 2000/13/ED – for permanent exemption from labeling. Request N° EFSA-Q-2007-002. Adopted on 15 October 2007 by written procedure.

(2) Food and Drug Administration (FDA). Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004. Congressional record v. 150  
<http://www.fda.gov/downloads/Food/LabelingNutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/UCM179394.pdf>

Figure 1. Immunoblot of DAS-44406-6 and Control (Maverick) Soybean Extracts with Soybean-Allergic Patient Sera



Lane	Contents
1	15 µL of control (Maverick) soybean seed extract (15µg)
2	15 µL of DAS-44406-6 soybean seed extract (15 µg)



Figure 2. ELISA Inhibition with DAS-444Ø6-6 and Control (Maverick) Soybean Extracts Using Soybean-Allergic Patient Sera

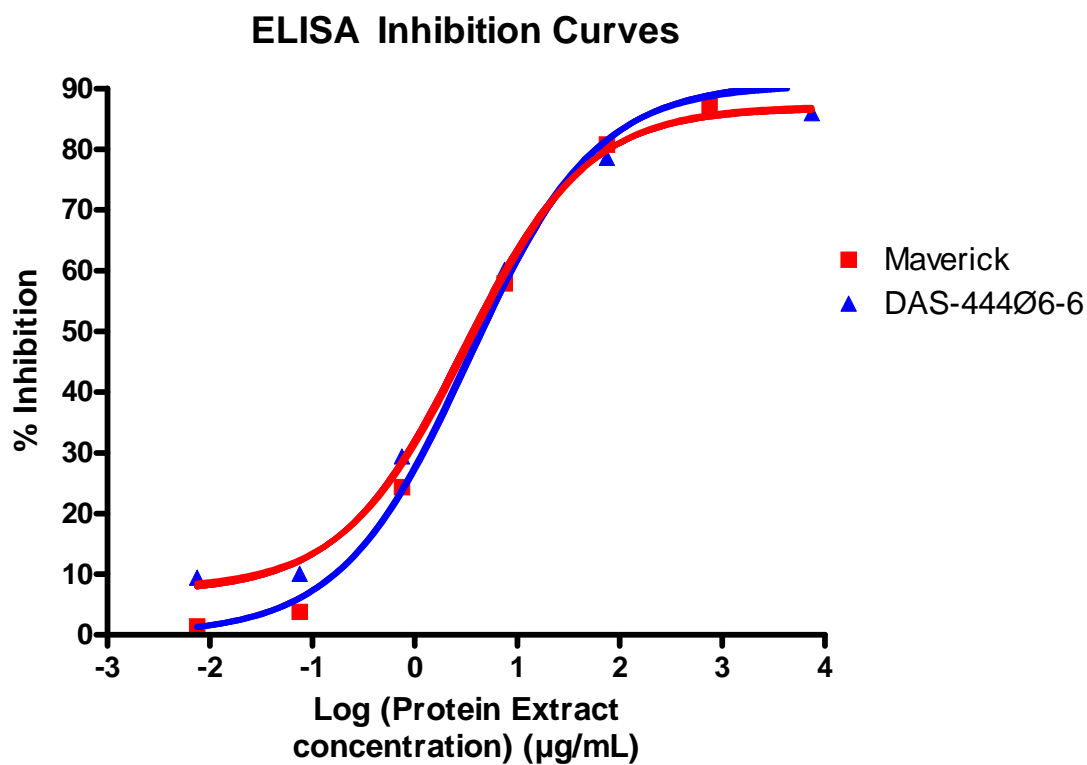
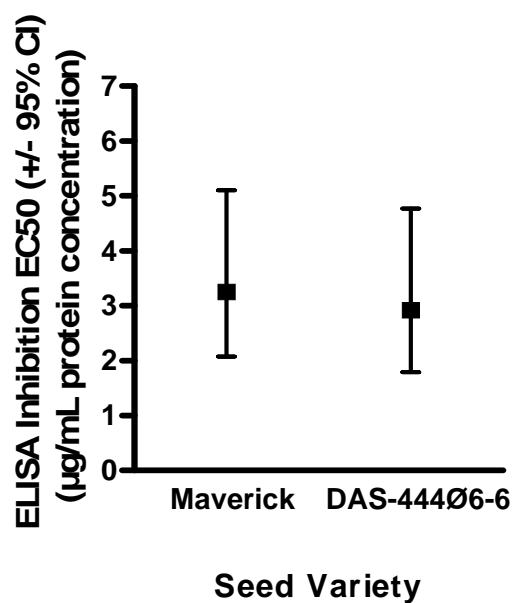


Figure 3. EC<sub>50</sub> values from the ELISA inhibition data for DAS-444Ø6-6 and Control (Maverick) Soybean Extracts and their 95% confidence intervals<sup>1</sup>

### EC<sub>50</sub>s Maverick Vs. DAS-444Ø6-6



<sup>1</sup> Note: Confidence limits are asymmetrical after transformation to the natural scale.