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STUDY TITLE

Endogenous Allergen Analysis of DAS-68416-4 Soybean

DATA REQUIREMENTS

Assessment of allergenicity of whole GM plant or crop

AUTHOR(S)

N.J. Stagg, Ph.D.

STUDY COMPLETED ON

February 4, 2010

PERFORMING LABORATORY

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

LABORATORY STUDY ID

101001

## Endogenous Allergen Analysis of DAS-68416-4 Soybean

SUMMARY

DAS-68416-4 soybean seeds were genetically modified in order to express the heterologous enzyme, aryloxyalkanoate dioxygenase-12 that confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate. 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-68416-4 soybean altered the endogenous allergen content of soybean. DAS-68416-4 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-68416-4 soybean and non-GM control (Maverick) are similar in endogenous allergen profiles. Therefore, the genetic modification used to generate DAS-68416-4 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

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Assessment of allergenicity of the whole GM plant or crop

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## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: DAS-68416-4

Title: Endogenous Allergen Analysis of DAS-68416-4 Soybean


- **STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS:**

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Company: Dow AgroSciences LLC

Company Agent: M. Krieger, Ph.D.

Title: Regulatory Manager

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Date: 4 Feb 2010

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

## STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

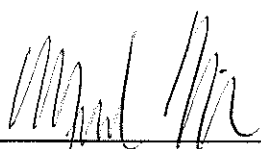
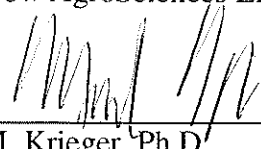
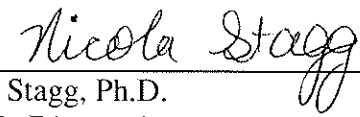
Title: Endogenous Allergen Analysis of DAS-68416-4 Soybean

Study Initiation Date: 10/01/2008

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	<u>4 Feb 2010</u> _____ Date
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 _____ N.J. Stagg, Ph.D. Study Director/Author Dow AgroSciences LLC	<u>4 Feb 2010</u> _____ Study Completion Date

## QUALITY ASSURANCE STATEMENT

Compound: DAS-68416-4

Title: Endogenous Allergen Analysis of DAS-68416-4 Soybean

Study Initiation Date: 10/01/2008

Study Completion Date: 02/04/2010

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## **NON-GLP STUDY**

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## Endogenous Allergen Analysis of DAS-68416-4 Soybean

### **ABSTRACT**

DAS-68416-4 soybean seeds were genetically modified in order to express the heterologous enzyme, aryloxyalkanoate dioxygenase-12 that confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate. 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-68416-4 soybean altered the endogenous allergen content of soybean. DAS-68416-4 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-68416-4 soybean and non-GM control (Maverick) are similar in endogenous allergen profiles. Therefore, the genetic modification used to generate DAS-68416-4 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

## INTRODUCTION

As soybean is one of the top eight important allergenic foods (Sampson, 1999; Sicherer and Sampson, 2006; Chapman *et al.*, 2006), a study was conducted to determine if the genetic modification used to generate DAS-68416-4 soybean altered the endogenous allergen content.

IgE binding to extracts of DAS-68416-4 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 20 clinically-reactive soy allergic patients [both children and adults with clinical histories of soybean allergy and CAP (Pharmacia Capsulated Hydrophobic Carrier Polymer) scores > 20].

Both Maverick and DAS-68416-4 soybean seeds were provided by Biotechnology Regulatory Science, Dow AgroSciences LLC, Indianapolis, IN. Human clinical sera were obtained from Plasma Lab International, Everett, WA, IBT Laboratories, Lenexa, KS and Duke University, Durham, NC.

## SDS-PAGE WITH COOMASSIE BLUE STAINING AND IMMUNOBLOT ANALYSIS

Extracts were prepared from the ground seed of DAS-68416-4 and Maverick soybeans. All samples were heated at 70 °C for 10 min and then run on SDS-PAGE with Coomassie blue to evaluate protein content of the two seed lots. Gel transfer of the proteins to a nitrocellulose membrane was performed with replicated blots, and one blot from each replicate was stained with Ponceau S stain to confirm protein transfer. Unstained blots were blocked in 2% BSA in PBST for at least 1 hour at room temperature followed by overnight incubation in serum from a pool of 20 soy-allergic patients held at 4 °C. Blots were washed with PBST to remove unbound IgE and then incubated in biotinylated goat IgG-anti-human IgE for 1 hr at room temperature with continuous agitation. Additional washing with PBST before and after adding NeutrAvidin-HRP conjugate was performed. Pierce SuperSignal chemiluminescent substrate was used for

development and visualization of the immunoreactive protein bands. The membranes were covered with Pierce SuperSignal reagent, exposed to Hyperfilm ECL in a darkroom and developed.

The protein profiles between DAS-68416-4 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-68416-4 and Maverick were compared in the one-dimensional immunoblot using soy-allergic sera and also showed no difference (Figure 1).

### **ELISA INHIBITION**

ELISA inhibition of IgE binding from a pooled soybean-allergic (20 patients) serum sample was conducted for DAS-68416-4 and control (Maverick) soybean extracts. Extracts from DAS-68416-4 and Maverick at various concentrations (0.004 to 4000 µg/ml of total soluble protein) were pre-incubated with the pooled serum and then transferred to 96-well plates that were previously coated with the non-transgenic control (Maverick) extracts (2 µg/well). After biotinylated goat IgG-anti-human IgE, NeutraAvidin-HRP conjugate and peroxidase substrate TMB additions with appropriate washing with PBST in between, plates were read on a microplate reader at 450 nm.

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:  $Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{(\text{LogEC50} - X) * \text{HillSlope}})}$ . 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 EC50 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 EC50 values and their associated 95% confidence intervals are plotted for the Maverick soybean and DAS-68416-4 extracts.

The ELISA inhibition data with the pooled soy-allergic serum showed the same IgE binding response between the non-transgenic Maverick soybean and DAS-68416-4 soybean extracts against 2 µg/well of immobilized Maverick extracts on the plate (

Figure 2). Furthermore, the associated EC50 values and 95% confidence intervals for Maverick and DAS-68416-4 were similar (Figure 3).

## CONCLUSION

The immunoblot and ELISA inhibition data demonstrate that the genetic modification used to generate DAS-68416-4 soybean did not alter the endogenous allergenicity compared with its non-transgenic control, Maverick.

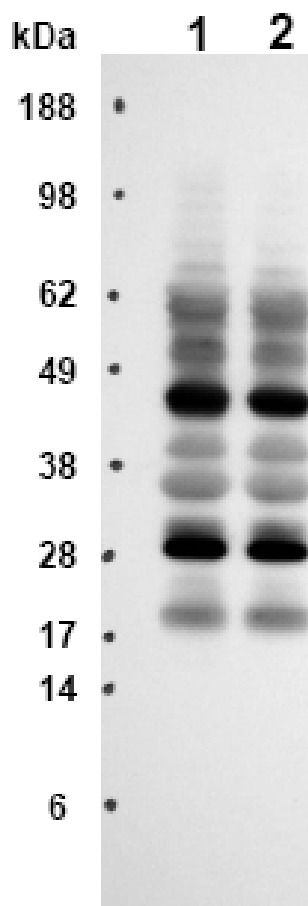
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Sicherer, S.H., Sampson, H.A. (2006) Food Allergy. *Journal of Allergy and Clinical Immunology*, 117:S470-S475.

Figure 1. Immunoblot of DAS-68416-4 and Control (Maverick) Soybean Extracts with Soybean-Allergic Patient Sera



Lane	Contents
1	15 µl of control (Maverick) soybean seed extract (10µg)
2	15 µl of DAS-68416-4 soybean seed extract (10 µg)

Figure 2. ELISA Inhibition with DAS-68416-4 and Control (Maverick) Soybean Extracts Using Soybean-Allergic Patient Sera

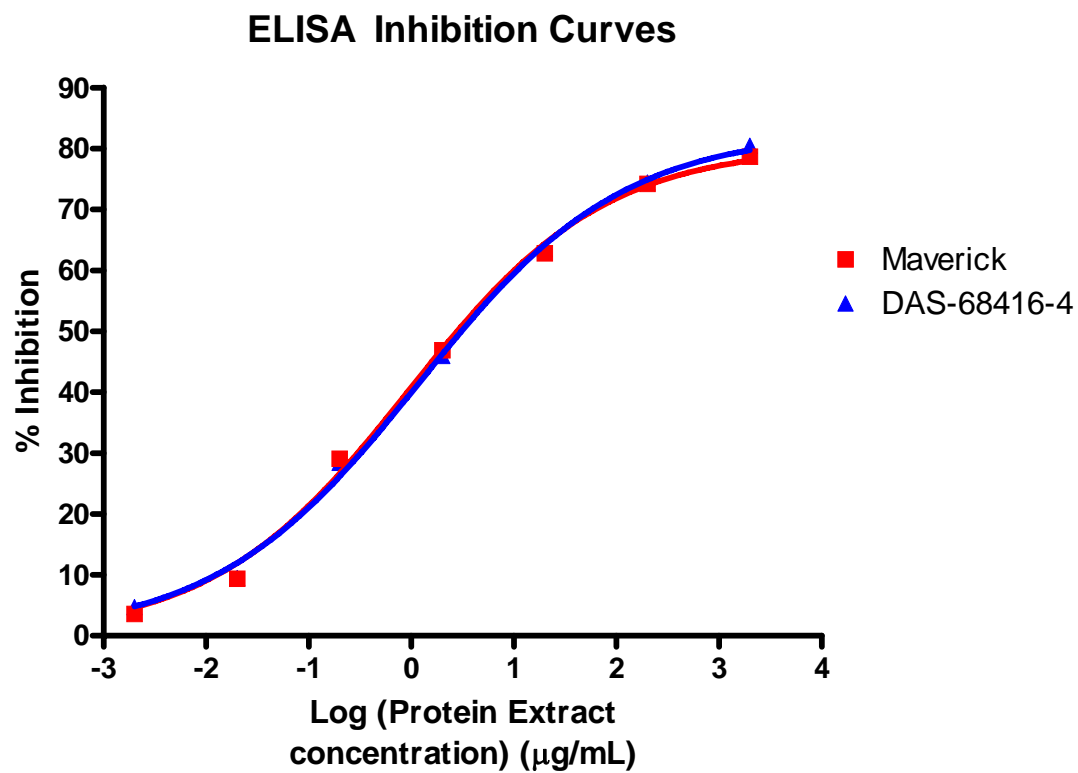
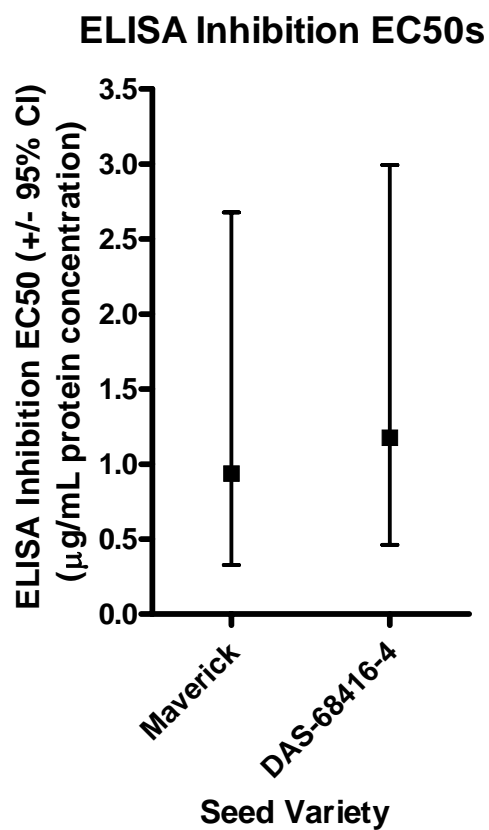




Figure 3. EC50 values from the ELISA inhibition data for DAS-68416-4 and Control (Maverick) Soybean Extracts and their 95% confidence intervals<sup>1</sup>



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