

SUMMARY

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STUDY TITLE

Potential Allergenicity Assessment of AAD-12 Protein Expressed in Soybean Event DAS-  
68416-4 by Bioinformatics Analysis (Update, March, 2010)

DATA REQUIREMENTS

N/A

AUTHOR(S)

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STUDY COMPLETED ON

23-April-2010

PERFORMING LABORATORY

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LABORATORY STUDY ID

101572

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N/A

AUTHOR(S)

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Compound: AAD-12 Protein

Title: Potential Allergenicity Assessment of AAD-12 Protein Expressed in Soybean  
Event DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

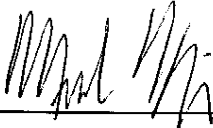
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Company Agent: M. S. Krieger

Title: Regulatory Manager

Signature: 

Date: 13 April 2010

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DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

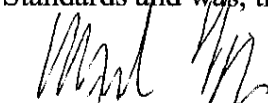
Study Initiation Date: 01/03/2010

This report represents data generated after the effective date of the 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

At the time this study was conducted, it was not subject to the Good Laboratory Practice Standards and was, therefore, not monitored by the quality assurance unit.




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22-April-2010  
Study Completion Date

## QUALITY ASSURANCE STATEMENT

Compound: AAD-12 Protein

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Event DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

Study Initiation Date: 01/03/2010

Study Completion Date: 04/23/2010

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## **NON-GLP STUDY**

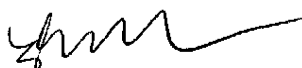
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Peer Reviewer  
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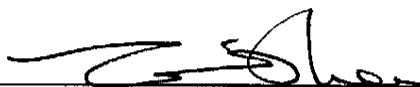
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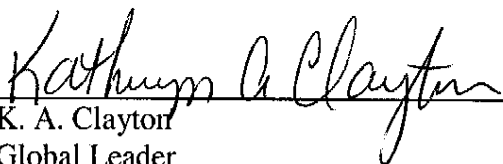
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## STUDY PERSONNEL

Title: Potential Allergenicity Assessment of AAD-12 Protein Expressed in Soybean  
Event DAS-68416-4 by Bioinformatics Analysis (Update, March, 2010)

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ABSTRACT

A plant-optimized *aad-12* gene, originally from common soil bacterium *Delftia acidovorana*, was integrated into Soybean (*Glycine max*) to produce event DAS-68416-4 by *Agrobacterium*-mediated transformation of a variety “Maverick” with plasmid pDAB4468, followed by conventional breeding. Aryloxyalkanoate dioxygenase-12 (AAD-12 protein), encoded by the *aad-12* gene, provides tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D), fluroxypyr, and triclopyr-based herbicides. The potential allergenicity of AAD-12 protein expressed in the soybean event DAS-68416-4 was evaluated using an updated allergen database (FARRP Allergen Database Version 10, Released January 2010) and bioinformatics tools. The AAD-12 protein sequence was queried this database to detect identities of >35% over 80 amino acids and matches of 8 contiguous amino acids with known allergens. The results indicate that the AAD-12 protein has no significant amino acid sequence similarities with known allergens.

## INTRODUCTION

A plant-optimized *aad-12* gene, originally from common soil bacterium *Delftia acidovorana*, was integrated into Soybean (*Glycine max*) to produce event DAS-68416-4 by *Agrobacterium*-mediated transformation of a variety “Maverick” with plasmid pDAB4468, followed by conventional breeding. Aryloxyalkanoate dioxygenase-12 (AAD-12 protein), encoded by the *aad-12* gene, provides tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D), fluroxypyr, and triclopyr-based herbicides.

In the safety assessment of transgenic crops, one of the concerns is that the introduced protein expressed in food crops might have a potential to elicit allergic reactions in human. Thus far, no single property of a protein is known to predict allergenic potential. For this reason, a weight-of-evidence approach to predicting allergenic risk has been adopted which considers multiple factors. To assess potential allergenicity of a protein, two criteria for evaluating structural similarities between query proteins and known allergens are currently used based on amino acid sequence alignments (1, 2, 3). The first criterion is a search over 80-amino-acid stretches (sliding window search) to detect >35% identity between a query protein and known allergens. The window size of 80 amino acids was selected to correspond with a typical domain size in a protein, and recognizes that single protein domains may contain epitopes that mediate antibody binding. The second criterion involves evaluating short amino-acid stretches for identity between the query protein and known allergens. As stated in the report of Codex Ad Hoc Working Group on Allergenicity (1), “the size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results”. Window sizes of 6 to 8 amino acids have been suggested based on hypothetical epitope sizes, however, use of window sizes of less than 8 amino acids have been largely abandoned based on the high probability of random alignments that are of no predictive value (4, 5). The use of any short-alignment criteria for predicting the allergenic potential of proteins has also been recently criticized (6, 7, 8, 9).

The purpose of this study was to evaluate the potential allergenicity of the AAD-12 protein expressed in soybean event DAS-68416-4 using bioinformatics tools and an updated allergen database.

## METHODS

### Query Sequence Preparation

The AAD-12 protein sequence was prepared in FASTA format accepted by FASTA and Fuzzpro search programs (Appendix 1).

### Allergenicity Assessment

For the allergenicity assessment, the amino acid sequence of the AAD-12 was compared with a peer-reviewed database containing 1471 known and putative allergens as well as celiac-induction proteins residing in the FARRP dataset (Version 10, Released in January 2010, University of Nebraska, <http://www.allergenonline.org>). Potential identities between the AAD-12 and proteins in the allergen database were evaluated with the FASTA program (v34) using the default algorithm parameters (Matrix = BLOSUM50; Expect = 10; Gap Penalties = -12/-2; ktup = 2).

The FASTA search was run by an in-house Perl script on a UNIX computer with a Linux operation system. If a query sequence is longer than 80 amino acids, the script parses the query sequence into a complete (overlapping) set of 80 amino acid long fragments and each fragment is subjected to a FASTA search. A greater than 35% identity threshold over any 80 or more amino acid sequences between a query sequence and an allergen was used to indicate the potential for cross-reactivity. To ensure that high identity over a short stretch (for example, 80% over 60 amino acids) will not be overlooked, a calculation,  $(\text{Identity}\% \times \text{number of overlapped amino acids})/80$ , was implemented as a conversion to check the criteria of >35% over 80 amino acids when the FASTA alignment (overlapped amino acids) is less than 80 amino acids. The AAD-12 protein sequence was also screened for any matches of 8 contiguous amino acids to the allergens contained in the database noted above. This was done using an in-house Perl script that

generates all sequentially possible (overlapping) 8-residue peptides from a query protein, followed by Fuzzpro program (Emboss Package v2.10.0) search that compares each query “word” with all allergen sequences in the database for perfect matches.

## RESULTS AND CONCLUSIONS

When the amino acid sequence of the AAD-12 protein was compared with the FARRP allergen dataset (Version 10), no over threshold identities (greater than 35% identity over greater than or equal to 80 amino acid residues) were detected in the FASTA search outputs (Appendix 2). No matches of eight or greater contiguous identical amino acids with known allergens in the database were observed in the entire AAD-12 sequence.

In conclusion, the results of this updated study show that AAD-12 does not share any significant amino acid sequence similarity with known protein allergens.

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## APPENDIX

### 1. Amino Acid Sequence of AAD-12

```
1  MAQTTLQITP  TGATLGATVT  GVHLATLDDA  GFAALHAAWL  QHALLIFPGQ
51  HLSNDQQITF  AKRFGAIERI  GGGDIVAISN  VKADGTVRQH  SPAEWDDMMK
101 VIVGNMAWHA  DSTYMPVMAQ  GAVFSAEVVP  AVGGRTCFADE  MRAAYDALDE
151 ATRALVHQRS  ARHSLVYSQS  KLGHVQQAGS  AYIGYGMDTT  ATPLRPLVKV
201 HPETGRPSLL  IGRHAHAIPG  MDAAESERFL  EGLVDWACQA  PRVHAHQWAA
251 GDVVVWDNRC  LLHRAEPWDF  KLPRVMWHSR  LAGRPETEGA  ALV
```

### 2. Archive of FASTA Search Output of AAD-12 Protein against Allergen Database V10

The AAD-12 protein was parsed into 214 fragments of 80-amino-acid long. Each of these fragments was queried against the allergen database using FASTA program. As such, output files with a combined size of more than thousands of pages were generated. These files are electronically stored in a secured computer in Dow AgroSciences and are available for viewing in PDF format.