

SUMMARY

(In accordance with 40 CFR part 152, this summary is available
for public release after registration)

STUDY TITLE

Seed Segregation of Soybean Event DAS-68416-4

DATA REQUIREMENTS

None

AUTHOR(S)

G. Shan and T. Hoffman

STUDY COMPLETED ON

15 November, 2010

PERFORMING LABORATORY

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

LABORATORY STUDY ID

102959

© 2010 Dow AgroSciences LLC All Rights Reserved.

This document is protected under copyright law. This document is for use only by the regulatory authority to which this has been submitted by the owners, and only in support of actions requested by the owners. Any other use of this material, without prior written consent of the owners, is strictly prohibited. By submitting this document, Dow AgroSciences does not grant any party or entity any right or license to the information or intellectual property described in this document

Seed Segregation of Soybean Event DAS-68416-4

SUMMARY

An F2 breeding generation of DAS-68416-4 soybean was planted and grown under field conditions to determine if the event followed the expected Mendelian inheritance pattern for a single gene. Plant segregation pattern was determined using a gene-specific fluorescence-based detection method specific for *aad-12*. The expected segregation ratio of 3:1 for plants containing *aad-12* (homozygous + hemizygous) versus plants not containing *aad-12* was observed.

STUDY TITLE

Seed Segregation of Soybean Event DAS-68416-4

DATA REQUIREMENTS

None

AUTHOR(S)

G. Shan 317-337-4118
[gshan@dow.com]
T. Hoffman

STUDY COMPLETED ON

15 November, 2010

PERFORMING LABORATORY

Regulatory Laboratories—Indianapolis Lab
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268-1054

LABORATORY STUDY ID

102059

© 2010 Dow AgroSciences LLC All Rights Reserved.

This document is protected under copyright law. This document is for use only by the regulatory authority to which this has been submitted by the owners, and only in support of actions requested by the owners. Any other use of this material, without prior written consent of the owners, is strictly prohibited. By submitting this document, Dow AgroSciences does not grant any party or entity any right or license to the information or intellectual property described in this document

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: DAS-68416-4 Soybean

Title: Seed Segregation of Soybean Event DAS-68416-4

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

Company: Dow AgroSciences LLC

Company Agent: M. Krieger

Title: Regulatory Manager

Signature: 

Date: 15 November 2010

*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

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

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Seed Segregation of Soybean Event DAS-68416-4

Study Initiation Date: 01-Dec-2008

Experimental Start Date: 05-Jan-2009

Experiment Termination Date: 30-Sept-2009

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

This study does not meet requirements of 40 CFR Part 160.

 _____ M. Krieger Sponsor Dow AgroSciences LLC	<u>15 November 2010</u> _____ Date
 _____ M. Krieger Submitter Dow AgroSciences LLC	<u>15 November 2010</u> _____ Date
 _____ G. Shan Author Dow AgroSciences LLC	<u>15 Nov. 2010</u> _____ Study Completion Date

QUALITY ASSURANCE STATEMENT

Compound: Aryloxyalkanoate Dioxygenase-12

Title: Seed Segregation of Soybean Event DAS-68416-4

Study Initiation Date: 01-Dec-2008

Study Completion Date:

NON-GLP STUDY

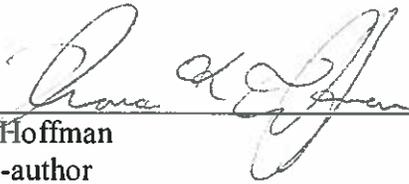
SIGNATURE PAGE



G. Shan
Author
Dow AgroSciences LLC

16 Nov. 2010

Date



T. Hoffman
Co-author
Dow AgroSciences LLC

11/16/10

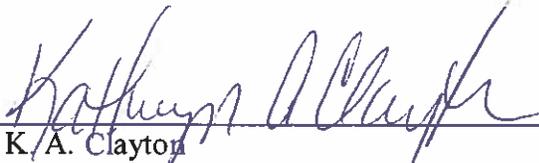
Date



C. Cui
Peer Reviewer
Dow AgroSciences LLC

11-16-2010

Date



K. A. Clayton
Global Leader, Biotechnology Regulatory
Science:
Dow AgroSciences LLC

16 Nov 2010

Date

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT.....	8
INTRODUCTION	9
METHODS AND MATERIALS.....	9
STUDY PERSONNEL	10
RESULTS	10
Table 1. Results of F2 individual plants tested for <i>aad-12</i> within a single segregating generation.	11
Appendix A. Statistical Analysis Result.....	12

Seed Segregation of Soybean Event DAS-68416-4

ABSTRACT

An F2 breeding generation of DAS-68416-4 soybean was planted and grown under field conditions to determine if the event followed the expected Mendelian inheritance pattern for a single gene. Plant segregation pattern was determined using a gene-specific fluorescence-based detection method specific for *aad-12*. The expected segregation ratio of 3:1 for plants containing *aad-12* (homozygous + hemizygous) versus plants not containing *aad-12* was observed.

INTRODUCTION

The segregation pattern of DAS-68416-4 soybean seeds from a F2 breeding generation was investigated to determine if the event followed the expected Mendelian inheritance pattern for a single gene.

METHODS AND MATERIALS

Test Materials

F2 seeds derived from crosses between event DAS-68416-4 T4 generation soybean plants expressing AAD-12 protein and elite soybean lines from Dow AgroSciences (DAS) R&D were planted at the DAS field station at Santa Isabel, Puerto Rico, USA in January 2009. After plants reached V4-6 stage, leaf samples were collected (8 leaf punches per plant) and shipped to DAS Indianapolis Lab, in Indiana, USA. The leaf samples were assayed for the presence or absence of *aad-12* using a gene-specific fluorescence-based detection method specific for *aad-12*.

List of Test Substances

Event name	Seed source IDs	Note
DAS-68416-4	GX08KX036881.001-6 GX08KX036901.001-6 GX08KX036929.001-6 GX08KX036949.001-6 GX08KX036977.001-6 GX08KX036997.001-6 GX08KX037025.001-6 GX08KX037045.001-6 GX08KX037073.001-6 GX08KX037093.001-6	F2 generation seeds

STUDY PERSONNEL

Analyst: Julissa Colon, Dow AgroSciences LLC

RESULTS

Chi-square analysis of trait inheritance data from a F2 breeding generation was conducted to determine the Mendelian inheritance of *aad-12* in DAS-68416-4 soybeans. The expected segregation ratio of 3:1 for plants containing *aad-12* (homozygous + hemizygous) versus plants not containing *aad-12* was observed.

Table 1. Results of F2 individual plants tested for *aad-12* within a single segregating generation.

Generation	Total plants tested	<i>aad-12</i> gene positive	<i>aad-12</i> gene negative	Expected ratio	P-value^a
F2	6774	5056	1718	3:1	0.492

^a Based on a chi-squared goodness of fit test

Appendix A. Statistical Analysis Result

```
TITLE1 'DAS STUDY 102059: Analysis';

PROC IMPORT DBMS=TAB OUT=work.data DATAFILE="C:\SASdatasets\aad12.data.txt"
REPLACE;
GETNAMES=YES;
QUIT;

PROC SORT DATA = data;
BY plant;
QUIT;

PROC FREQ DATA = data;
TABLES result / CHISQ TESTP=(0.25 0.75);
QUIT;
```

DAS STUDY 102059: Analysis
14:03 Thursday, November 11, 2010

The FREQ Procedure

result	Frequency	Percent	Test Percent	Cumulative Frequency	Cumulative Percent
0	1718	25.36	25.00	1718	25.36
1	5056	74.64	75.00	6774	100.00

Chi-Square Test
for Specified Proportions
Chi-Square 0.4726
DF 1
Pr > ChiSq 0.4918

Sample Size = 6774