

SUMMARY

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

STUDY TITLE

AAD1 Amino-Acid Homology Search for Similarity to Toxins

DATA REQUIREMENTS

None

AUTHOR(S)

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STUDY COMPLETED ON

26-Feb-2007

PERFORMING LABORATORY

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LABORATORY STUDY ID

071022

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SUMMARY

The AAD1 (aryloxyalkanoate dioxygenase 1) protein was evaluated for amino-acid sequence similarity to known toxins. A global sequence similarity search of this protein sequence against the GenBank non-redundant protein dataset was conducted in an effort to identify potential safety concerns by examining the characteristics of related proteins. The AAD1 similarity search identified 581 proteins. None of the similar proteins returned by the search identified safety concerns that might arise from the expression of AAD1 in plants.

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: AADI

Title: AADI Amino-Acid Homology Search for Similarity to Toxins

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: P. L. Hunst

Title: Global Regulatory Trait Leader

Signature: Denny G. Hunst

Date: 2/23/2007

*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: AAD1 Amino-Acid Homology Search for Similarity to Toxins

Study Initiation Date: 02/10/2007

Experimental Start Date: 02/10/2007

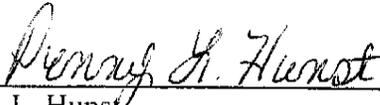
Experiment Termination Date: 02/19/2007

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

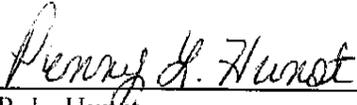
This study does not meet requirements of 40 CFR Part 160.



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2/26/2007

Study Completion Date

QUALITY ASSURANCE STATEMENT

Compound: AAD1

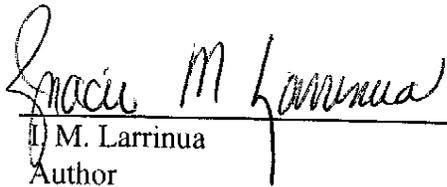
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NON-GLP STUDY

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AAD1 Amino-Acid Homology Search for Similarity to Toxins

ABSTRACT

The AAD1 (aryloxyalkanoate dioxygenase 1) protein was evaluated for amino-acid sequence similarity to known toxins. A global sequence similarity search of this protein sequence against the GenBank non-redundant protein dataset was conducted in an effort to identify potential safety concerns by examining the characteristics of related proteins. The AAD1 similarity search identified 581 proteins. None of the similar proteins returned by the search identified safety concerns that might arise from the expression of AAD1 in plants.

INTRODUCTION

The AAD1 (aryloxyalkanoate dioxygenase 1) protein was evaluated for amino-acid sequence similarity to known toxins. A global sequence similarity search of this protein sequence (Figure 1) against the GenBank non redundant protein dataset was conducted in an effort to identify potential safety concerns by examining the characteristics of related proteins. This data base was posted on February 10th, 2007 and had 4,554,902 sequences with 1,568,234,006 amino acids.

METHODS AND MATERIALS

The similarity search was conducted using the BLASTP 2.2.10 algorithm (1) with a cutoff expectation (E) value of 1.0. AAD1 is an enzyme and preliminary blast results indicated that there would be more than 500 significant hits which is the limit of the web based blast interface. Therefore blastp was run on the command line using the following set of parameters –
`/usr/local/blast-2.2.10/bin/blastall -p blastp -d /usr/local/blast-2.2.10/db/blastlibs/nr -i /genomics/AAD1.txt -o /genomics/AAD1results01292007 -e 1 -v 5000 -b 5000 -F ""`

RESULTS AND DISCUSSION

The AAD1 similarity search identified 581 proteins. These proteins were annotated with an enzyme function, either because the blast description line itself contained a well defined enzymatic activity, or if no well defined enzyme activity appeared in the description line, because the GenPept page for this protein contained a well characterized enzyme activity in its Features annotation under “region_name”. Five hundred and eighty proteins were identified as alpha ketoglutarate-dependent dioxygenases which is not surprising since AAD1 is an alpha ketoglutarate-dependent dioxygenase. These proteins can be broken down into a few major subclasses. The largest class was the taurine catabolism dioxygenases with 478 members (2). The next largest class with 52 members were the alpha-ketoglutarate-dependent 2,4-

dichlorophenoxyacetate dioxygenases (3). Thirdly, with 44 members, were clavaminic acid synthetases (4). There were 5 alkylsulfatases (atsK) (5). AAD1 itself is a *R*-2,4-dichlorophenoxypropionate dioxygenase (6). Many of these proteins also had a tauD annotation as taurine catabolism dioxygenases. The last protein was a hypothetical protein with no functional annotation. Inspection of the BLink results accessible from the GenPept page of this protein reveals significant homology ($8e-21$) to a heavy myosin from *Cryptosporidium hominis* TU502.

CONCLUSIONS

None of the similar proteins returned by the search identify safety concerns that might arise from the expression of AAD1 in plants.

REFERENCES

1. Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. *J. Mol. Biol.* 215:403-10.
2. Eichhorn E., Van Der Ploeg J. R., Kertesz M. A., Leisinger T. Characterization of alpha-ketoglutarate-dependent taurine dioxygenase from *Escherichia coli*. *J. Biol. Chem.* 272: 23031-23036 (1997)
3. Suwa Y, Wright A. D., Fukimori F.120 , Nummy K. A. , Hausinger R. P. , Holben W. E. , Forney L. J. Characterization of a chromosomally encoded 2,4-dichlorophenoxyacetic acid/alpha-ketoglutarate dioxygenase from *Burkholderia* sp. strain RASC. *Appl. Environ. Microbiol.* 62: 2464-2469 (1996)
4. Zhang Z., Ren J. S., Stammers D. K., Baldwin J. E., Harlos K., Schofield C. J.; Structural origins of the selectivity of the trifunctional oxygenase clavaminic acid synthase.; *Nat. Struct. Biol.* 7:127-133(2000).
5. Muller I, Kahnert A, Pape T, Sheldrick G. M, Meyer-Klaucke W, Dierks T, Kertesz M, Uson I. Crystal structure of the alkylsulfatase AtsK: insights into the catalytic mechanism of the Fe(II) alpha-ketoglutarate-dependent dioxygenase superfamily. *Biochemistry.* 2004 Mar 23;43(11):3075-88
6. Schleinitz, K. M., Kleinsteuber, S., Vallaey, T. and Babel, W. Localization and Characterization of Two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy)propionate Cleavage in *Delftia acidovorans* MC1.

FIGURE 1. AMINO ACID SEQUENCE OF AAD1

MAHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAF
HTYQVIYFPGQAITNEQHIAFSRRFGPVDVPLLKSIEGYPEVQMIRREA
NESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVPEHGGDTGFLSMYTAWET
LSPTMQATIEGLNVVHSATRVFGSLYQAQNRRFSNTSVKVMVDAGDRET
VHPLVVTHPGSGRKGLYVNQVYCQRIEGMTDAESKPLLQFLYEHATRFDF
TCRVRWKKDQVLVWDNLCTMHRVDPDYAGKFRYLTRTTVGGVRPAR