US EPA ARCHIVE DOCUMENT

State of California **Department of Pesticide Regulation**

EVALUATION REPORT – PESTICIDE

Date: May 20, 2004

Microbiology – Hanna Daoud

Product Name	:	*Aspergillus flavus* AF36
I.D. No.	:	205526-N
Applicant	:	Arizona Cotton Research and Protection Council
EPA Reg. No.	:	71693 - 1
Document No.	:	52965 - 0001, - 0002, - 0012
Active Ingredient	:	*Aspergillus flavus* strain AF36
Use	:	For displacing aflatoxin-producing-fungi on cotton
Registration Action	:	Section 3 / New Active Ingredient
Area of Review	:	Microbiology

Registration Specialist : Gary Sprock / John Inouye

[ ] Data/Information Support Registration

[x] Data/Information Support Conditional Registration

[ ] Data/Information Do Not Support Registration

[ ] No Registration Action Required

Summary:

On behalf of the Arizona Cotton Research and Protection Council, the Interregional Research Project No.4 (IR-4), submitted an application for the registration of the Microbial Pest Control Agent (MPCA) *Aspergillus flavus* AF36, EPA Reg. No. 71693-1 for displacing/reducing aflatoxin-producing strains of *Aspergillus flavus* on cotton in California.

Aspergillus flavus AF36 is a solid granular end-use product formulation containing 0.0008% by weight (3000 CFU/g) *Aspergillus flavus* strain AF36, a non-aflatoxin-producing (atoxigenic) strain of the fungus *Aspergillus flavus*, as the active ingredient.

The product is to be broadcast on soil surface as a prebloom application once a year either by ground application or by aerial application at the rate of 10 pounds end-use product (which is equivalent to 0.0008 pound of active ingredient) per acre. The product label recommends that the colonized wheat seeds (the end-use product) not be covered with soil, and it cautions that cultivation after aerial and ground application may diminish product efficacy. The label also recommends that within 3 days of application, the fields are to be irrigated to promote germination of the fungal strain AF36 which is expected to colonize the cotton crop and soil before the aflatoxin-producing strains of *Aspergillus flavus* proliferate, thus, reducing aflatoxin contamination of cotton and cotton seeds.

The product has been conditionally registered by the U.S.EPA for this use on cotton in the states of Arizona and Texas only, effective June 24, 2003. This conditional registration is contingent upon compliance with the conditions and timeframes specified in EPA's Notice of Conditional Registration dated June 24, 2003.

Now, the registrant, Arizona Cotton Research and Protection Council, is seeking to add the state of California to the EPA's registered label and has submitted information on Product Chemistry/Microbiology to address the following:

- Product Identity and Composition (Record No. 209910, Document No. 52965-0012).
- Manufacturing Process and Quality Control (Record No. 209911, Document No. 52965-0012).
- Discussion on Formulation of Unintentional Ingredients (Record No. 209912, Document No. 52965-0012).
- Analytical Methods.
- Certified limits (per EPA's CSF).
- U.S.EPA's "Biocide Registration Action Document for *Aspergillus flavus* AF36 (PC Code 006456)". (DPR Record No. 209899, Document No. 52965-0001).

The submitted data/information have been reviewed and a number of deficiencies have been identified. These deficiencies are outlined in the "Conclusion" portion of this report.

It is to be noted that these deficiencies have also been identified in U.S.EPA review of the subject product and were made conditions for the existing EPA Conditional Registration in the states of Arizona and Texas. They will also be applicable to California.

Discussion:

Based on the information provided, the subject product, *Aspergillus flavus* AF36, is an end-use product. This end-use product basically consists of sterile wheat seeds that have been colonized by the fungus *Aspergillus flavus* strain AF36, a non-aflatoxin-producing (atoxigenic) strain of *Aspergillus flavus* which constitutes the active ingredient in the product. The sterile wheat seed serve as a carrier and as a nutrient source for the living fungus.

The product label and CSF show the product contains 0.0008% by weight (3000 CFU/g) *Aspergillus flavus* strain AF36 as the active ingredient. Analytical data demonstrating/ substantiating this claimed value for the active ingredient in the product have not been provided.

Aspergillus flavus strain AF36, the active ingredient in the subject product, is said to be a naturally occurring (not genetically modified) atoxigenic strain of *Aspergillus flavus* whose species are ubiquitous worldwide. It was isolated from cottonseed collected in the Yuma Valley of Arizona. The isolate has been deposited at the American Type Culture Collection under the

accession numbers ATCC – 96045 and ATCC – 96047. It is reported to be distinguishable from aflatoxin-producing *Aspergillus flavus* strains by vegetative compatibility analysis. It is not clear whether this analytical method is capable of distinguishing strain AF36 from other strains of *Aspergillus flavus* including other non-aflatoxin-producing strains.

Strain AF36 has been characterized as an "atoxigenic" strain of *Aspergillus flavus* for its lack of aflatoxin production. Aflatoxin is a potent carcinogen produced by toxigenic strains of *Aspergillus flavus*. Its levels in cotton and cotton byproducts, cottonseed oil and cottonseed meal, are regulated by the FDA.

It is to be noted that the inability to produce aflatoxin does not necessarily mean the inability to produce other mycotoxins or other harmful or undesirable metabolites. Therefore, the capacity of strain AF36 to produce other metabolites needs to be assessed.

Detailed description of the manufacturing process and a brief discussion of the quality control program employed during the manufacturing process, as well as a brief discussion on the formation of unintentional ingredients have been provided. However, supporting data from sample analysis and quality control tests demonstrating product purity, quality and integrity were not provided. Also not provided are the quality control standards (acceptance/rejection limits) to be employed in the production process. Also, data from storage stability study were not provided.

A temporary exemption from the requirement of a residue tolerance of *Aspergillus flavus* AF36 on cotton was established in 40 CFR 180.1206 in conjunction with the Experimental Use Permit 69224 EUP-1 in Arizona, with an expiration date of December 30, 2003. For the current EPA 3(C)(7)(C) conditional registration in Arizona and Texas, a permanent tolerance exemption is being established in 40 CFR 180.1206 for residue of *Aspergillus flavus* AF36 on cotton when used as labeled as a prebloom application.

Aspergillus flavus AF36 exemption from the requirement of a residue tolerance negates the need for a residue-enforcement analytical method. However, analytical methods for product characterization and quality control during the manufacturing process and for product quality enforcement at the marketing level are still required.

Due to the nature and composition of this microbial product, many of the physical and chemical properties required of chemical pesticides, including pH, melting point, boiling point, solubility, vapor pressure, dissociation constant, octanol/water partition coefficient, oxidizing or reducing potential, flammability/flash point, explodability, viscosity, corrosion characteristics, and dielectric breakdown voltage, are not applicable to this product; therefore, they are not required.

Competitive exclusion is reported to be the mode of action or mechanism by which strain AF36 replaces aflatoxin-producing *Aspergillus flavus* strains. Upon application, the level of strain AF36 increases during the postapplication germination phase, but this increase returns to normal within a few weeks.

The displacement of the aflatoxin-producing strains by strain AF36 does not appreciably change the total *Aspergillus* population. However, it may reduce the aflatoxin-producing strains and thereby reduces aflatoxin contamination of cotton seed. Even if AF36 does not control or reduce aflatoxin levels in treated cotton seed, it is not likely to increase it to above normal background levels.

The application of a single, seasonal, prebloom application at the proposed low rate of 10 pounds end-use product (equivalent to less than 0.01 pound active ingredient) per acre is not likely to appreciably change the overall quantity of *Aspergillus flavus* spores in the environment, or to increase aflatoxin to above normal background levels within the range of natural variation in cotton seed or in the environment. Therefore, its impact on the environment, if any, is likely to be minimal or negligible.

In its review as expressed in EPA "Biocide Registration Action Document", the U.S.EPA found *Aspergillus flavus* AF36 to be **eligible for a conditional registration**, and that its proposed use on cotton in Arizona and Texas to be in the "**public interest**", and that AF36 is not likely to pose undesirable risk to health or the environment, and that certain conditions apply to this eligibility and the applicant must take certain action (e.g. generate and provide certain data) within the timeframe outlined in section VI titled "Action Required by Registrant" (page 45 of 55 pages, Record No. 209899).

Also in its "**Public Interest Finding**", the U.S.EPA indicated that the Agency believes the use of AF36 under this conditional registration would be in the public interest according to the criteria for public interest outlined in 51 FR No.43, Wednesday March 5, 1986. And stated that: "The Agency has determined that the conditional registration of the indigenous *Aspergillus flavus* AF36 is likely to provide a cost effective biocontrol agent for reduction of aflatoxin in cotton and its food/feed by products, and is in the public interest." (Pages 38 of 55 pages, U.S.EPA Biocide Registration Action Document for *Aspergillus flavus* AF36, Record No. 209899).

Conclusion:

The submitted data and information support a conditional registration of the subject product for the proposed use on cotton in California, provided the applicant agrees to submit the following required data/information within one year from the date the conditional registration becomes effective:

1. Results of five-batch analysis study demonstrating:
   a. Number of colony forming units (CFU) of *Aspergillus flavus* strain AF36 per gram product.
   b. Certification of limits.
   c. Identity and number of potential microbial contaminants (human pathogens).
   d. Identity and quantity of metabolites and other unintentional ingredients.
2. Storage stability.
3. Quality control standards (acceptance/rejection limits) for the active ingredient, potential microbial contaminants, harmful metabolites, if any, and other unintentional ingredients that may be present in the product.
4. Method for identifying/distinguishing strain AF36 from other strains of *Aspergillus flavus* (including other non-aflatoxin-producing strains) other than the vegetative compatibility method.
5. An enforcement analytical method for purposes of enforcing product quality at the market-place level.

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State of California | Department of Pesticide Regulation

EVALUATION REPORT - PESTICIDE

Date: April 1, 2004

ID : 205526-N
EPA Reg No : 71693-1 (new a.i.)
Applicant : IR-4
Registrant : Arizona Cotton Research and Protection Council
Active Ingredient : *Aspergillus flavus* strain AF36
Use : Fungicide
Registration Action : Section 3
Area of Review : Fish and Wildlife (Endangered Species)

Registration Specialist: John Inouye

[X] Data support registration

[ ] Data does not support registration

[ ] No registration action required

SUMMARY: In accordance with Title 3, Section 6206 of the California Code of Regulations (CCR), the director is considering a petition by the Interregional Research Project Number 4 (IR-4) for Section 3 registration of a non-aflatoxin producing strain of *Aspergillus flavus* on cotton to control aflatoxin-producing strains of *Aspergillus flavus* on cotton. The proposed use calls for surface broadcast treatments of cultures grown on wheat.

Pursuant to CCR Sections 6158(d) and 6206, this review considers use limitations to mitigate adverse effects to non-target organisms with particular attention to protection of federally listed species.

TOXIC POTENTIAL: *Aspergillus flavus* is ubiquitous in the environment and is exempt from tolerance. Fish and wildlife hazard data has been waived by USEPA because the proposed use will not significantly increase the naturally occurring populations of *A. flavus*.

CONCLUSION: There is a low probability of exposure to fish and wildlife generally from the proposed use in cotton and low toxic potential indicated by natural occurrence of the organism and waivers of toxicology data requirements. Due to low toxic potential and low probability of exposure, use of this product per label directions is not likely to pose risks to fish or wildlife in general nor to listed species in particular.

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R142233

Chemical: Aspergillus flavus 36 colonized wheat seed

PC Code:
006456

HED File Code: 41500 BPPD Tox/Chem
Memo Date: 5/20/2004
File ID: 00000000
Accession #: 000-00-9002

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