



US Environmental Protection Agency Office of Pesticide Programs

Biopesticide Registration Action Document

Aspergillus flavus AF36

PC Code 006456

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***Aspergillus flavus* AF36**

PC Code 006456

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
BIOPESTICIDES AND POLLUTION PREVENTION DIVISION

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I. EXECUTIVE SUMMARY/FACT SHEET

Active Ingredient and Proposed Use

The active ingredient (ai), *Aspergillus flavus* AF36, also referred to as AF36 (PC Code 006456), belongs to the naturally occurring genus of fungi, *Aspergillus*, which are ubiquitous in the environment. The non-aflatoxin-producing L strain, AF36, was isolated in Arizona (AZ), and is also found in Texas (TX). Its lack of vegetative compatibility with aflatoxin-producing strains is a trait used to screen starter cultures for production of the pesticide. Because of this trait, AF36 is not likely to exchange genetic material with the toxigenic *A. flavus* strains. Prebloom applications of AF36 are expected to displace the aflatoxin-producing strains of *A. flavus* from the cotton crop and fields.

Starter cultures of AF36 are maintained in pure culture and routinely checked to ensure the lack of aflatoxin producers. Analysis of aflatoxin is performed after extraction and analyzed by standard thin layer chromatography (tlc) procedures and visualization via scanning fluorescence densitometry scanning. Other appropriate methods are required for quality control of the pesticide to assure product characterization, the control of human pathogens and other unintentional metabolites or ingredients within regulatory limits, and to ascertain storage stability and viability of the pesticidal active ingredient. All cotton and its byproducts must meet regulatory levels for aflatoxin as required by the Food and Drug Administration (FDA).

Toxicology, Human Exposure and Risks

Evaluations of mammalian toxicology data comply with the Food Quality Protection Act (FQPA) of 1996, and are sufficient to support the conditional registration of this microbial pesticide for the proposed uses. The pesticide is categorized as Toxicity Category IV for acute oral toxicity. Acute pulmonary toxicity studies demonstrate a low toxicity potential for AF36. An acute inhalation study was not required, pursuant to 40 CFR§158.740(c), because the granular End-use Product (EP) consists of approximately 99% sterilized inoculated wheat seeds, which are not likely to contain respirable particles of less than 10 microns [Table 2a, Section III.B.2]. Based on the acute pulmonary study and the nature of the inerts, AF36 is considered Toxicity Category III for acute inhalation toxicity effects.

The Agency has accepted the rationales to waive data for primary dermal irritation, primary eye and skin irritation, acute dermal toxicity/pathogenicity, acute intraperitoneal, and the hypersensitivity study. The rationales for the data waiver requests, were based on (a) low toxicity potential as demonstrated by acute oral and pulmonary infectivity/toxicity studies; (b) soil and air monitoring studies over several years to demonstrate that exposure to AF36 is not above background *A. flavus* levels; (c) lack of pesticide drift based on the granular nature of the EP and agricultural application methods; (d) known characteristics of the genus *Aspergillus*; and (e) no documented reports of hypersensitivity incidents associated with the use of the pesticide during the research, manufacture and experimental phases [Table 2b and discussion in Section III.B.2].

Food Tolerances

This is the first proposed conditional registration for the subject active ingredient, which has been used during research trials under an Experimental Use Permit (EPA# 69224-EUP-1) from 1996 to present. A temporary exemption from the requirement of a tolerance for residues of AF36 on cotton was established in 40 CFR 180.1206 in connection with the EUP. For this Section 3(c)(7)(C) conditional registration, a permanent tolerance exemption is being established in 40CFR 180.1206 for residues of AF36 on cotton, when used as labeled as a prebloom application and as discussed in this document.

FQPA Considerations

The Agency has considered AF36 in light of the safety factors of the Food Quality Protection Act (FQPA) of 1996 and has made a determination of reasonable certainty of no harm to the U.S. population in general, and to infants and children in particular. The ubiquitous occurrence of *Aspergillus* strains suggest that the fungus is normally expected to be present in/on food commodities regardless of treatment with AF36. Thus, applications of AF36 are not expected to increase the exposure to *A. flavus* strains above normal background levels [Section **III.B.3**].

No toxicity endpoints were indicated to justify setting a numerical tolerance for AF36. Based on submitted studies, AF36 demonstrates low acute oral Toxicity Category IV potential, indicating no incremental dietary risk above that which currently exists to *Aspergillus flavus* strains. Cotton itself is not a direct food commodity and potential transfer of residues of AF36 to edible cotton food/feed commodities is not likely. Residues of AF36, the microbial active ingredient, are not expected to survive the heating and pressure associated with the processing of cottonseed into cottonseed meal. Neither AF36 nor aflatoxin are likely to separate into the edible fraction, cottonseed oil. Thus, dietary exposure via cottonseed oil and secondary transfer of AF36 residues to meat and milk via cottonseed meal are not likely to be above background levels [Section **III.B.3**].

The Agency also considered the potential contamination of AF36 by aflatoxin, a metabolite of the aflatoxin-producing strains of *A. flavus*, and required quality control and quality assurance methods be in place to ascertain integrity of the pesticide. According to submitted studies, starter cultures of AF36 are to be screened by thin layer chromatography and scanning fluorescence densitometry for lack of aflatoxin. Batches with potential contaminants above regulatory levels are to be destroyed. Levels of aflatoxin in cotton and its byproducts, cottonseed oil and cottonseed meal are regulated by the FDA [Section **III.B.3**].

In this assessment no acute, subchronic, chronic, immune, endocrine, or nondietary exposure issues have been identified which may have any incremental adverse effects on infants, children and the general U.S. population. Based on the Toxicology Category IV for acute oral toxicity, and Toxicity Category III for acute inhalation toxicity effects, a safety factor is not required for residues of AF36. The potential for transfer of AF36 residues to human adults,

infants and children via dietary exposure is not likely to be greater than exposure to current existing levels of *A. flavus* strains [Section III.B. 2, 3 & 6].

Dietary exposure and risk are not likely to be greater than that which normally exists to the naturally occurring *Aspergillus* fungal strains [Section III.B.3]. Potential risks via exposure to drinking water or runoff are adequately mitigated by, among other things, percolation through soil [Section III.B.5]. There is a potential for aggregate non-occupational dermal, and inhalation exposures of adult humans, infants and children to the microbe because of the ubiquitous distribution of *Aspergillus* fungi in the environment. However, residential exposure to the AF36 colonized wheat seeds is not likely to exceed those background levels of *A. flavus*, because the pesticide is applied with minimal drift and at very low rates to commercial, agricultural sites [Section III.B.7]. There are no documented reports of hypersensitivity incidents during the 6 years of research, manufacture, and experimental use of AF36 [Section III.B.1.d.&e]. Furthermore, there is no indication that the fungus, *A. flavus* AF36, shares any common mechanisms of toxicity with other registered microbial fungal active ingredients to affect cumulative exposure and risk to this pesticide [Section III.B.8]. Thus, exposure to adult humans, infants and children, from the proposed use of AF36 is not likely to pose any incremental risk above that which currently exists from exposure to the naturally occurring *A. flavus* strains.

Occupational and Residential Exposure and Risk

Potential exposure of AF36 to workers and pesticide handlers is not expected to pose any undue risk. Pesticide drift is minimized because the End-use Product (EP) consists of large granules, and cultivation is not recommended after aerial and ground application. Appropriate Personal Protective Equipment (PPE) and a Restricted-entry Interval (REI) of 4 hours are required to mitigate any potential risks to workers and pesticide handlers. Residential exposure and risk are not expected to be above background *A. flavus* levels, because the pesticide demonstrates low toxicity potential and it is to be applied to commercial, agricultural sites. [Section III.B.4].

Ecological and Environmental Exposure and Risks

Evaluations of avian infectivity/pathogenicity and honeybee data indicate that the toxicological effects of the pesticide are not likely to pose any incremental adverse concerns to non-target organisms. The justifications to waive test data for freshwater fish, estuarine and marine vertebrates and invertebrates, and terrestrial non-target plants, which are discussed more fully in Section III.C.1.c, are acceptable for the proposed uses, based on low exposure scenarios. While data were waived for most non-target insects, an acceptable study demonstrated low potential toxicity/pathogenicity effects to honey bees and other pollinators [Section III.C.1].

Aspergillus flavus strains are ubiquitous around the world. As expected, levels of AF36 increase during the postapplication germination phase, but the slight increase returns to normal within a few weeks. Even though the total *Aspergillus* population does not increase, AF36 displacement of the toxigenic strain may reduce the environmental burden of aflatoxin-producing

strains of *A. flavus*. Soil and air monitoring data, collected during the experimental phase, demonstrate the efficacy of AF36 to displace the toxigenic aflatoxin-producing strains in AZ [Section **III.D**]. As a condition of registration, the Agency is requiring efficacy data from large scale applications in TX.

Based on the low toxicity/pathogenicity potential demonstrated in the data evaluated, the low doses to be used, and the ubiquitous nature of the microorganism, no incremental risks are expected to non-target organisms, if AF36 is used as labeled.

Data Gaps and Requirements/Labeling

All deficiencies and labeling must meet Agency requirements [Section **IV.C**]. Standard analysis of 5 production batches and efficacy data from large scale trials in Texas are required as conditions of registration [Section **VI**]. If more extensive use patterns are sought for treatment of other agricultural terrestrial sites or crops, additional information and data will be required on a case by case basis.

II. OVERVIEW

A. Product Overview

Biological Name:	<i>Aspergillus flavus</i> AF36
ATCC Number:	96045
Trade and Other Names:	<i>Aspergillus flavus</i> AF36; AF36.
OPP Chemical Code:	006456
Basic Manufacturer:	Arizona Cotton Research and Protection Council, 3721 East Wier Avenue Phoenix, Arizona 85040-2933

B. Use Profile

The following is information on the proposed uses with an overview of use sites and application methods.

Type of Pesticide:	Fungicide
Use Sites:	Cotton in Arizona and Texas
Target Pests:	Reduction of the aflatoxin-producing strains of <i>Aspergillus flavus</i>
Formulation Types:	Solid, granular (colonized wheat seeds)

Method and Rates of Application: Apply 10 pounds End-use Product by air or through a cultivator mounted granular applicator. This is equivalent to much less than 0.01 lb active ingredient per acre or per 13,000 linear feet based on 40 inch rows. Cultivation may diminish efficacy. Do not cover the granules with soil. Furrow irrigate the crop with at least 2 inches of water within three days after application.

Use Practice Limitations: For use on cotton only in Arizona and Texas

Timing: prebloom application once a year

C. Estimated Usage

Estimates based on existing commercial use cannot be made since this is the first conditional registration of this active ingredient. The antifungal agent has been used in an Experimental Use Permit (EPA Reg. No. 69224-EUP-1) since May 1996, with the current extension scheduled to expire December 31, 2004. Usage of the End-use product (EP) during the experimental period was projected to be at 10 pounds per acre. Acreages treated during the EUP ranged from 1,000 to 22,000 acres per year over the permitted research period 1996-2004. Approximately 673,809 pounds of the End-use Product were applied over 1996 to 2002 during the EUP. Projected usage for 2003 is 200,000 pounds EP. Label claim for the EP is 0.0008 percent active ingredient. Thus, the **total active ingredient** usage from 1996 to 2003 is calculated to be approximately 6.99 pounds.

D. Data Requirements

The submissions to comply with Agency data requirements for granting this conditional registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). For AF36, the product identity and analysis data, as well as the information submitted for acute mammalian toxicology and ecological effects are sufficient to allow the proposed use patterns. Based on evaluations of the submitted data and information, as discussed in this document, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of AF36, as long as it is used as labeled.

Conditions of registration for this new active are analyses from 5 production batches to include:

- (i) certifications of limits;
- (ii) identification of *A. flavus* AF36 by either DNA analysis or some other method different from the vegetative compatibility method now in use;
- (iii) analysis and quantification of metabolites and other unintentional ingredients;
- (iv) identification and enumeration of potential human pathogens;
- (v) storage stability; and
- (vi) viability data;

In addition, efficacy data are required from large scale trials in Texas. If more extensive use patterns are sought for treatment of other agricultural terrestrial sites or crops, additional information and data will be required on a case by case basis.

E. Regulatory History

Experimental Use and Temporary Tolerance Exemption

Notices of a receipt of application and the filing of a pesticide petition for the use of a new active ingredient, *Aspergillus flavus* AF36, in an experimental program were published in the Federal Register [FR: February 28, 1996. Vol. 61, No. 40, page 7512]. These applications were filed by the Interregional Research Project No. 4 (IR-4), New Jersey Agricultural Experiment Station, Technology Center, 681 U.S. Highway 1 South, North Brunswick, NJ 08902-3390, on behalf of the Southern Regional Research Center, United States Department of Agriculture, Agricultural Research Service (USDA ARS), 1100 Robert E. Lee Blvd., New Orleans, LA 70179-0687. On June 14, 1996, the Agency established a temporary exemption from the requirement of a tolerance for use of AF36 on cotton [FR: June 14, 1996. Vol. 61, No. 116, page 30235]. The use of this active ingredient is consistent with an Experimental Use Permit, EPA Reg. No. 69224-EUP-1 and with Pesticide Petition (PP) 5E4575. AF36, a non-aflatoxin-producing strain of *A. flavus*, was to be applied prebloom as an antifungal agent to displace the aflatoxin-producing strains present in or on the cotton crop and soil in cotton fields.

The first site of application in 1996 for the 3-year EUP was 1000 acres in Arizona (AZ). Later, the EUP was extended to December 30, 2001, and to include treatment of 20,000 acres in AZ. The temporary exemption from the requirement of a tolerance was concurrently amended to comply with the requirements of the Food Quality Protection Act (FQPA of 1996) [FR: May 26, 1999. Vol. 64, No. 101, page 28371; FR: June 30, 1999. Vol. 64, No. 125, page 35049]. During these extensions, the registrant continued to generate acute mammalian toxicological, non-target avian and honeybee, and efficacy data to fulfil Agency requirements. A further extension was granted for both the temporary exemption from the requirement of a tolerance and the EUP to increase the acreage to be treated to 22,000 and to include Texas (TX) [FR: July 17, 2002. Vol. 67, No. 137, page 46884]. The EUP and the temporary exemption from the requirement of a tolerance (40 CFR 180.1206) will expire on December 31, 2004. No adverse effects have been reported in the annual EUP progress reports submitted to the Agency. The Experimental Use Permit and the exemption from temporary tolerance will no longer be applicable when the conditional registration and the permanent exemption from tolerance take effect.

Section 3 Registration and Exemption from tolerance

EPA received an application from Interregional Research Project No. 4 (IR-4), New Jersey Agricultural Experiment Station, Technology Center, 681 U.S. Highway 1 South, North Brunswick, NJ 08902-3390 on behalf of the Arizona Cotton Research and Protection Council, 3721 East Wier Avenue Phoenix, Arizona, to register the active ingredient, *Aspergillus flavus* AF36. The pesticide is to be applied prebloom, by air and ground equipment, to cotton fields in AZ and TX. USDA ARS has allowed the Arizona Cotton and Research Council use of the data for AF36 which were obtained from the studies during the Experimental Use Permit, EPA Reg. No. 69224-EUP-1. When the application package was deemed complete, the receipt of the

application for the new active ingredient was published in the Federal Register [FR: March 12, 2003. Vol. 68, No. 48, page 11841].

Concomitant with the application for the Section 3(c) registration, the registrant filed a petition (PP 8E5001) requesting a permanent exemption from the requirement of a tolerance for the active ingredient, *Aspergillus flavus* AF36, a non-aflatoxin-producing strain of *A. flavus*, on cotton and its food/feed commodities. A notice of filing of this petition was published in the Federal Register [FR: February 14, 2003. Vol. 68, No. 31, page 7554]. Several comments, mainly from cotton growers, processors, and ginners in AZ and TX, were received during the comment period for the application. These comments reiterated their support of the use of AF36 on cotton. An exemption from the requirement of a tolerance for residues of AF36 in/on cotton is being processed in connection with this petition, and the final rule will be published in the Federal Register (40CFR§180.1206), concurrent with the conditional registration. This conditional registration and the exemption from tolerance for residues of *Aspergillus flavus* AF36 supersede the Experimental Use Permit and the temporary exemption from tolerance.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

The data submitted in support of product identity requirements for AF36 are sufficient for the proposed use patterns of the microbial pesticide.

1. Product Identity and Mode of Action

Product Identity

Aspergillus flavus AF36 (also referred to as AF36) is a non-aflatoxin-producing or atoxigenic strain of *Aspergillus flavus*, which is ubiquitous around the world. Some members of the genus *Aspergillus* produce mycotoxins, such as aflatoxin, a potent carcinogen produced by toxigenic strains of *A. flavus*. Other members of the genus *Aspergillus* have been domesticated for commercial use. For example, products for human consumption include “beano” which contains alpha-galactosidase obtained from *Aspergillus niger*, and soy sauce and miso, fermentation products derived from the action of *Aspergillus oryzae*.

The Agency has classified AF36 as an active ingredient for use in microbial pesticides. The non-aflatoxin-producing L strain of the *Aspergillus flavus* fungus is a naturally occurring strain that was isolated in Arizona from cottonseed, and it also is indigenous to Texas. AF36 is identified by its lack of aflatoxin production and its unique vegetative compatibility group which may not allow exchange of genetic material with the aflatoxin-producing strains. Sterilized wheat seeds are colonized with the AF36 fungus and kept in appropriately labeled containers prior to application.

2. Physical And Chemical Properties Assessment

Product identity and manufacturing data support the conditional registration of *Aspergillus flavus* AF36 (Table 1a). Identification of AF36 (non-aflatoxin-producing strain) is verified on the basis of vegetative compatibility. Starter cultures are monitored for aflatoxin production by standard thin layer chromatography (tlc) procedures and visualization via scanning fluorescence densitometry [MRID 44626101; BPPD Review, March 29, 1999, (hereinafter referred to as “BPPD Review - March 29, 1999”)]. There is a zero tolerance for aflatoxin-producing strains based on these techniques.

Starter cultures are also screened for coliforms on Violet Red Bile (VRB) Agar, and for bacteria by plating on nutrient agar [MRID 43763402; BPPD Review dated May 14, 1999, (hereinafter referred to as “BPPD Review - May 14, 1999”); MRID 44626101; BPPD review - March 29, 1999). Batches with contamination, such as metabolites of concern, human pathogens, and unintentional ingredients, above quality assurance levels must be destroyed.

The pesticide has the color of, and looks and smells like, wheat seeds (Table 1b). The inert ingredient for the End-use Product, sterilized wheat seed, which serves as a matrix for the inoculant, is exempt from the requirement of a tolerance under 40 CFR 180.950(a) and is cleared for food use.

Guideline data requirements (40 CFR §158.740(a)) for melting point, boiling point, solubility, vapor pressure, dissociation constant, octanol/water partition coefficient, stability, oxidizing or reducing potential, flammability/flash point, explosability, viscosity, miscibility, and dielectric breakdown voltage were waived because of the nature of the microbial pesticide.

As a condition of registration, further characterization is required from 5 production batches to include:

- (i) certifications of limits;
- (ii) identification of *A. flavus* AF36 by either DNA analysis or some other method different from the vegetative compatibility method now in use;
- (iii) analysis and quantification of metabolites and other unintentional ingredients;
- (iv) identification and enumeration of potential human pathogens;
- (v) storage stability; and
- (vi) viability data.

Table 1a: Product Identity & Manufacturing Process for *Aspergillus flavus* AF36

Guideline	Study	Result	MRID #
151-10 *885.1100	Product Identity	Isolated from cottonseed, Yuma desert, AZ. Acceptable	43763401
151-11 *885.1200	Manufacturing Process	Acceptable	43763401 44597001 44713701
151-12 *885.1300	Discussion of Formation of Unintentional Ingredients	Acceptable for experimental batches. Microbial contamination, aflatoxin levels are examined for quality control. Data on production batches required as a condition of registration. See Section VI.	43763402 44626101
151-13 *885.1400	Analysis of Samples	Acceptable for experimental batches. Spores quantified by turbidimetry. Standard curve relates turbidity of spore suspension to viability (cfu). Data on production batches required as a condition of registration. See Section VI.	44626101 43972403**
151-15 *885.1500	Certification of limits	Acceptable for experimental batches. Data on production batches required as a condition of registration. See Section VI.	44626101
151-16	Analytical Method	Acceptable for experimental batches. Vegetative compatibility for fungal active ingredient starter cultures. Aflatoxin analyzed by tlc and scanning fluorescence densitometry. Further strain characterization by DNA analysis or other appropriate method required as condition of registration.	44626101

*OPPTS Harmonized Guidelines **cross reference, acute oral study, Table 2a.

Table 1b: Physical & Chemical Properties of *Aspergillus flavus* AF36

Physical/Chemical Properties			
Guideline	Study	Result	MRID #
151-17	color	color of wheat seeds.	43763401
	physical state	looks like wheat seeds	43763401
	odor	smells like wheat seeds	43763401

B. Human Health Assessment

1. Food Clearances/Tolerances

This is the first proposed Section 3(c)7(C) conditional registration of the subject strain, *Aspergillus flavus* AF36. It has been used in the field under Experimental Use Permit 69224-EUP-1, during which time a temporary exemption from the requirement of a food tolerance was established and extended to December 30, 2004 (40 CFR §180.1206). Residues of AF36 or its metabolites are not expected on the food/feed commodity, cotton. Aflatoxin is a potential metabolite of the aflatoxin-producing strains but not of AF36. All cotton products are subject to compliance with the regulatory levels of aflatoxin as regulated by the Food and Drug Administration.

There is a reasonable certainty that no harm is likely to result from exposure to AF36. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. Below is the toxicology assessment, and discussion of other factors under the Food Quality Protection Act (1996), which led to the decision regarding the exemption from tolerance for residues of AF36 to be granted concomitant with the conditional registration of the pesticide (40 CFR §180.1206).

2. Toxicology Assessment

Mammalian toxicology studies have been submitted and are sufficient to support the conditional registration of the microbial pesticide for the proposed use patterns. Summaries of the acute toxicological studies (Table 2a) and the rationales for certain data waiver requests (Table 2b) are discussed below.

a. Acute Oral Toxicity (MRID 43972403; OPPTS 885.3050)

Five male and 5 female Sprague Dawley rats were treated with the microbial pesticide (500 mg/ml or 6.3×10^3 cfu/ml) by gavage (MRID 43972403; BPPD Data Evaluation Report, Acute Oral Toxicity Study in Rats, dated April 23, 1996, (hereinafter referred to as "BPPD Review - April 23, 1996")). During the observation period at 2 and 4 hours post dosing, and daily for 14 days thereafter, 1 female lost body weight (bw) from day 1 to day 8. Other rats gained body weight throughout the study. All rats were examined by necropsy for any macroscopic abnormalities at the end of the study. No clinical signs or abnormalities were noted during the study, and the pesticide was considered to be neither pathogenic nor infective following oral administration of a single dose. With an LD₅₀ greater than 5000 mg/kg body weight, the pesticide was classified as Toxicity Category IV for acute oral effects.

Table 2a: Tier I - Acute Mammalian Toxicity of *Aspergillus flavus* AF36

Guideline	Study	Toxicity Category	Results	MRID #
152-10 *885.3050	Acute oral toxicity/ pathogenicity	IV	Acceptable. LD ₅₀ > 5000 mg/kg. 5 male, 5 female Sprague Dawley treated 500 mg/ml or 6.3 x 10 ³ cfu/ml.	43972403
152-32 *885.3100	Acute inhalation	III	Pursuant to 40 CFR sec. 158.740(c), because the majority of the aerodynamic equivalent of the product is not composed of particles less than 10 microns in diameter, an inhalation study was not required. Nevertheless, this requirement was considered satisfied based on clearance observed in the acute pulmonary study.	45798201
152-32 *885.3150	Acute pulmonary toxicity/ pathogenicity	N/A	Acceptable. AF36 not toxic, infective or pathogenic via intratracheal instillation to rats. Clearance by day 8.	45739101 45798101 45798201

* OPPTS Guideline Numbers.

b. Acute Pulmonary Toxicity/Pathogenicity (MRID 45798201; OPPTS 885.3150)

Three studies were submitted in support of the mammalian acute infectivity/pathogenicity pulmonary guideline: a range finding study and two complete acute pulmonary infectivity/toxicity studies. The dose-range study [MRID 45739101; BPPD Data Evaluation Record, dated April 02, 2003a, (hereinafter referred to as “BPPD Review - April 02, 2003a”)] concluded that 10⁸ cfu/rat would be a suitable test dose level for the acute pulmonary infectivity/toxicity studies.

The first complete acute pulmonary infectivity/toxicity study was conducted with Tween 80 as a surfactant in the test material. Results from this study indicated that the test organism was neither infective nor pathogenic, in spite of rat mortality, which may have been due to the detergent. A second complete study, without Tween 80, was considered acceptable and demonstrated no toxicity for pulmonary effects. Both of these studies, summarized below, complied with the Good Laboratory Practices (GLP) of the United Kingdom (UK) and the Organization for Economic Cooperation and Development (OECD) and are scientifically acceptable for the purpose of registration.

In the first acute pulmonary toxicity study, 26 male and 26 female Sprague Dawley rats, approximately 8 to 10 weeks old, were used. Test animals were each dosed with a single intratracheal dose of 1.2 ml at 5.30×10^8 cfu/ml (or 1.28 to 1.63×10^8 cfu/animal) [MRID 45798101; BPPD Review, Data Evaluation Record, April 02, 2003b, (hereinafter referred to as “BPPD DER - April 02, 2003b”)]. Spores from the colonized sterilized wheat seeds were harvested in sterile distilled water containing 0.5% Tween 80. The test material was administered in 0.1% sterile physiological saline in 0.1% Tween 80. Transient clinical signs and mortality were observed in rats. The study author indicated that the etiology of the deaths of 14 rats by day 4 of the study is not clear, and may be due to dosing the test organism in Tween 80 causing “a severe acute inflammatory response leading to death.”

Body weights of the surviving rats were recorded on days 1 (prior to dosing), 4, 8, 15, 22. Brain, spleen, liver, lymph nodes, heart, lungs, and cecum of sacrificed rats were examined *post mortem*. *Aspergillus flavus* AF36 was detected in lungs, cecal contents, and feces on day 4 with clearance by day 8 after dosing. No test organisms were detected in any samples from the shelf control and inactivated test organism treated rats.

The acceptable second complete pulmonary toxicity study was a repetition of the initial pulmonary test, but was conducted without Tween 80 [MRID 45798201; BPPD Review, Data Evaluation Record, dated April 02, 2003c, (hereinafter referred to as “BPPD DER - April 02, 2003c”)]. It was UK and OECD GLP compliant, except for test substance characterization, stability and homogeneity. Pre-dose and post-dose suspensions were 9.67×10^8 cfu/ml and 1.03×10^8 cfu/ml, respectively. Each of the 25 male and 25 female Sprague Dawley rats (approximately 8 to 10 weeks old) received a single intratracheal dose of approximately 1.2ml. Mortality of 4 rats by day 2 appeared to be attributable to an initial dosing effect. The rest of the test animals showed an initial response but then rapid recovery indicating no toxicity. Although some surviving rats lost weight intermittently, all surviving rats gained weight prior to scheduled sacrifice.

No clinical signs that were considered to be due to the test organism were observed in the test rats. Organs were examined *post mortem* as previously described. *Aspergillus flavus* AF36 was detected in the lungs with clearance by day 8 after dosing. No test organism, *A. flavus* AF36, was detected in any samples from the shelf control or inactivated test organism treated rats. Therefore, based on the presented/submitted data, *A. flavus* AF36 was not toxic to rat pulmonary systems [MRID 45798201; BPPD Review - April 02, 2003c]. The study is ACCEPTABLE.

c. Acute Inhalation (OPPTS Guideline 152-32)

The inert is sterilized wheat seeds, which acts as a matrix and nutrient source for the germinating AF36. These sterilized wheat seeds, comprising more than 99% of this pesticidal

product, do not contain respirable particles greater than 10 microns. For this reason, and because

Table 2b: Tier I - Data Waivers: Acute Mammalian Toxicity of *Aspergillus flavus* AF36

Guideline	Study	Toxicity Category	Comments	MRID No.
152-31 *885.3100	Acute dermal toxicity	N/A	Waived**	N/A
152-33 *885.3200	Intraperitoneal injection toxicity/pathogenicity	N/A	Waived** No toxicity observed during acute oral and acute pulmonary studies as discussed above.	43972403 45739101 45798101 45798201
152-35 *870.2400	Primary eye irritation	N/A	Waived**	N/A
152-34 *870.2500	Primary dermal irritation	N/A	Waived** <i>Aspergillus</i> genus contains some known dermal sensitizers. Low exposure and application to commercial sites indicate minimal/negligible potential for non-occupational residential dermal exposure. In absence of data for AF36, label accordingly to mitigate occupational exposure. Low exposure and any potential pesticide drift can be mitigated with appropriate PPE.***	N/A
152-36 *870.2600	Dermal sensitization			

*OPPTS Harmonized Guideline Numbers. ** Justifications acceptable, see text.

***See Labeling Section IV.D.

e. Data Waiver Requests: Health Effects

Data waivers were requested for the following Tier I studies:

- (i) Acute Dermal Toxicity/Pathogenicity (OPPTS 885.3100)
- (ii) Primary Dermal Irritation (OPPTS 870.2500)
- (iii) Primary eye irritation (OPPTS 870.2400)
- (iv) Intravenous, Intracerebral, Intraperitoneal injection (OPPTS 885.3200)
- (v) Hypersensitivity study (Guideline 152-36)
- (vi) Immune response (Guideline 152-38)

The Agency decided that the justifications provided by the applicant to waive the studies listed above, [(i) through (vi)], were acceptable as discussed below [BPPD review of Data Waiver Requests....AF36 for use on cotton...dated May 22, 2003 (hereinafter referred to as "BPPD memo - May 22, 2003)].

Summaries of discussions for Data Waiver Requests

- (i) Acute Dermal Toxicity/Pathogenicity (OPPTS 885.3100)**
- (ii) Primary Dermal Irritation (OPPTS 870.2500)**
- (iii) Primary eye irritation (OPPTS 870.2400)**

With regards to the dermal and eye irritation guideline tests, it was impractical to apply the End-use Product, sterilized wheat seeds inoculated with *Aspergillus flavus* AF36, as test material. Furthermore, non-occupational dermal and eye exposures, or exposures via any of the routes in (i) thru (vi) above, are not likely to be above background levels of the naturally occurring *A. flavus*, as discussed below.

1. *Aspergillus*, a saprophytic fungus, is a normal constituent of the microflora in air and soil. The naturally occurring soil and plant colonizer is also found on living and dead plant material throughout the world. Aflatoxin-producing strains of *Aspergillus flavus* are particularly prominent in hot, dry climates supplemented with irrigation and are ubiquitous components of the natural Arizona desert ecosystem. Populations of *A. flavus* typically increase during crop production and the fungus occurs widely on crop debris left in the soil. Shortly after application, AF36 germinates, displacing the aflatoxin-producing strains from cotton and the soil, and spore levels return to background. This was demonstrated in soil and air monitoring studies submitted over multiple years of experimental usage [MRIDs 45307201, 45307202: BPPD Review of Soil and Air Monitoring Studies and Product Performance Testing (Efficacy), dated May 15, 2003, (hereinafter referred to as "BPPD review - May 15, 2003")].

2. The proposed label rate is low, being much less than 0.01 lb active ingredient in 10 pounds End-use Product per acre, and commercial, agricultural sites are treated, thus minimizing non-occupational dermal exposure at residential sites. A low application rate indicates that incremental exposure is not likely to be greater than that which occurs normally to naturally occurring *Aspergillus flavus* strains [BPPD review - May 15, 2003].

3. Drift is not expected during application based on the large granular nature of the pesticide (i.e. sterilized inoculated wheat seeds). In addition, since only 1 prebloom application is made, and cultivation is not recommended after application, the potential for non-occupational dermal and residential exposure is unlikely.

(iv) Intravenous, Intracerebral, Intraperitoneal Injection (OPPTS 885.3200)

Submitted acute oral and pulmonary toxicity/pathogenicity studies in the rodent (required for microbial pesticides) indicate that following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the active ingredient. The acute oral toxicological study (Toxicity Category IV) demonstrated an LD₅₀ of greater than 5000 mg/kg body weight with no toxicity/infectivity effects, and demonstrable clearance from organs examined post mortem [MRID 43972403; BPPD Review - April 23, 1996]. Organs were examined *post mortem* as previously described [Section III.B.2.a & b.] *Aspergillus flavus* AF36 was detected in the lungs with clearance by day 8 after dosing. No test organism, *A. flavus* AF36, was detected in any samples from the shelf control or inactivated test organism treated rats. The acceptable acute pulmonary study, and the non-respirable nature of the inerts, were used to categorize the pesticide as Toxicity Category III for acute inhalation effects [MRID 45798201; BPPD Review - April 02, 2003c]. The results from these rodent studies support waiving the data requirement for the acute Intravenous, Intracerebral, Intraperitoneal Injection (OPPTS 885.3200).

(v) Hypersensitivity study (Guideline 152-36)

A hypersensitivity study was waived since hypersensitivity incidents were not reported from maximally exposed workers and researchers during the research and experimental phases associated with the use of the active ingredient, *A. flavus* AF36 [BPPD Review - April 02, 2003d; see Section III.B.d above]. Nevertheless, reports of hypersensitivity incidents associated with the use of the pesticide are still required to comply with FIFRA 6(a)(2) requirements.

(vi) Immune response (Guideline 152-38)

Rodent studies submitted in support of *Aspergillus flavus* AF36 indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the active ingredient [BPPD Review - April 23, 1996; BPPD Review -

April 02, 2003c; Section **III.B.2.a & b.**] Thus, the data waiver request for immune response is granted for the proposed use of AF36 on cotton.

On the basis of the foregoing rationales, and there being no documented problems associated with the non-aflatoxin producing strain, *Aspergillus flavus* AF36, data waivers for the following studies were granted for the proposed use of *Aspergillus flavus* AF36 on the food/feed commodity, cotton in Arizona and Texas: **(i) Acute Dermal Toxicity/Pathogenicity; (ii) Primary Dermal Irritation; (iii) Primary eye irritation; (iv) Intravenous, Intracerebral, Intraperitoneal Injection (OPPTS 885.3200); (v) Hypersensitivity study (Guideline 152-36); and (vi) Immune response (Guideline 152-38).** These conclusions may be revisited if other application methods, uses, or sites are requested for *Aspergillus flavus* AF36, or adverse effects are reported in connection with the use of AF36.

f. Subchronic, Chronic Toxicity and Oncogenicity

Based on the data generated in accordance with the Tier I data requirements (40 CFR §158.740(c)), Tier II tests (Guidelines 152B-40 through 152B-49) involving acute oral, acute inhalation, subchronic oral, acute intraperitoneal/intracerebral, primary dermal, primary eye, immune response, teratogenicity, virulence enhancement, and mammalian mutagenicity were not required. As a result, Tier III tests (Guidelines 152-50 through 53) involving chronic testing, oncogenicity testing, mutagenicity, and teratogenicity also were not required.

g. Effects on the Immune and Endocrine Systems

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate.” Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen and thyroid systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC’s recommendation that the program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

The Agency is not requiring information on the endocrine effects of this active ingredient, *Aspergillus flavus* AF36, at this time. The Agency has considered, among other relevant factors, available information concerning whether the microorganism may have an

effect in humans similar to an effect produced by a naturally occurring estrogen or other endocrine effects. There is no known metabolite that acts as an "endocrine disrupter" produced by this microorganism. The submitted toxicity/pathogenicity studies in the rodent (required for microbial pesticides) indicate that following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the active ingredient. In addition, based on the low potential exposure level associated with the proposed single, seasonal prebloom application of the pesticide, the Agency expects no incremental adverse effects to the endocrine or immune systems.

3. Dietary Exposure and Risk Characterization (includes drinking water)

Dietary Exposure

The proposed food use pattern is not likely to result in dietary exposure or residues on food and feed. Cotton is not itself a direct dietary commodity and AF36 can be found on cotton seed. Residues of AF36, the microbial active ingredient, are not likely to survive the heating and pressure associated with the processing of cottonseed into cottonseed meal. Moreover, AF36 will not separate into the edible fraction, cottonseed oil. Thus, potential transfer of residues of AF36 to edible cotton food/feed commodities is not expected. Consequently, human dietary exposure to AF36 via cottonseed oil, or by secondary transfer of AF36 residues to meat and milk via cottonseed meal, is unlikely to be above naturally occurring background levels. Dietary exposure via drinking water, as presented below (**see 5**), does not pose an incremental risk.

Based on submitted studies, the pesticide End-use Product, *Aspergillus flavus* AF36, demonstrates low acute oral toxicity category IV potential [BPPD Review - April 23, 1996]. No toxicity endpoints were indicated to justify setting a numerical tolerance for the fungal active ingredient, *Aspergillus flavus* AF36. An LD₅₀ greater than 5000 mg/kg body weight in the acute oral studies discussed above, indicates that consumption of food commodities treated with AF36 poses no incremental risk via dietary exposure. Indeed, the submitted data indicate no toxicity or infectivity of AF36 in the acute oral test mammalian systems. Therefore, the Agency has determined that dietary exposure to AF36 is not likely to result in any undue health effects or risk.

While the Agency has concluded that AF36 is not likely to add to the dietary burden, any potential contribution by AF36 to aflatoxin contamination was also considered for a conservative estimate of the health effects of this pesticide. This is because aflatoxin is considered a public health hazard, and AF36 is proposed as a biocontrol agent for aflatoxin-producing strains of *A. flavus* (*see Section III.D.*). Even if AF36 does not control aflatoxin levels in the treated cotton food/feed commodities, cotton and its by products are screened for aflatoxin prior to their introduction into the channels of commerce. For instance, FDA does not allow cottonseed products containing aflatoxin above 20 parts per billion (ppb) to be used in dairy rations, or above 300 ppb to be used for feeding beef cattle.

As previously stated, the registrant claims that quality control and selection procedures will not allow aflatoxin production in the starter cultures for pesticide manufacture [BPPD review - March 29, 1999; BPPD review - May 14, 1999]. Any batches with aflatoxin or aflatoxin-producing *Aspergillus flavus* are to be destroyed. For these reasons, the Agency has determined that use of AF36 will not add to the dietary burden of aflatoxin, but is more likely to ameliorate aflatoxin levels in treated cotton food/feed commodities. Therefore, dietary exposure to aflatoxin, as a result of AF36 use, will not be greater, but may even be less, than that which currently exists.

4. Occupational and Residential Exposure and Risk Characterization

a. Occupational Exposure

Dermal exposure via the skin would be the primary route of exposure for mixer/loader applicators. The pesticide belongs to the genus, *Aspergillus*, many members of which are known to be dermal sensitizers. While it is not known whether this strain is more or less likely than other *A. flavus* strains to induce hypersensitivity, no hypersensitivity incidents have been reported during the 14 year laboratory research phase or the 3 to 6 year field research and experimental phase. During aerial application, dermal exposure is most likely to be greater to mixer/loaders and flaggers than to applicators, who pilot aircraft. Dermal exposure and risk are likely to occur to mixer/loaders and applicators during ground treatment. However, the rate of application is low, much less than 0.01 pound of active ingredient in the 10 pounds End-use Product applied per acre. There is only 1 prebloom application per growing season. The label specifically recommends against cultivation of fields, thus minimizing dermal exposure. Drift and, consequently, worker exposure are minimized because of the granular nature of the pesticide, i.e. inoculated sterilized wheat seeds. Environmental expression studies done during the multiple year EUP showed that, while population levels increased slightly after application, there were no significant increases in the total exposure to *A. flavus* over the season.

Appropriate labeling is required to protect mixer/loaders, flaggers, applicators and postharvest workers who are likely to be exposed to the pesticide. Workers and pesticide handlers are required to wear the following PPE: long sleeved shirt, long pants, shoes, socks, gloves, goggles and appropriate respirator. A Restricted-Entry Interval (REI) of 4 hours is required following application of the pesticide. Early-entry workers must wear coveralls in addition to the PPE above during the REI to perform post-application activities. If the pesticide is used as labeled, the potential for occupational dermal exposure and risk is expected to be minimal.

b. Residential, School and Day Care Exposure and Risk Characterization

The evaluation of acute pulmonary toxicity mammalian data resulted in a categorization of the pesticide as Toxicity Category III for effects associated with inhalation exposure [BPPD Review - April 02, 2003c]. As described elsewhere, pesticide drift is expected to be minimal based on the granular nature of the pesticide, inoculated sterilized wheat seeds. In addition, use sites will be commercial and agricultural. Moreover, in soil and air monitoring studies to assess AF36 efficacy, slight increases in spore levels shortly after application returned to normal background levels [BPPD Review - May 15, 2003]. Thus, incremental exposure and risk to infants, children and adults to AF36 is not expected to be significantly greater than background levels of *A. flavus*. Furthermore, it must be kept in mind that *Aspergillus flavus* strains are ubiquitous, and the use of the AF36 strain to displace the toxigenic strain may minimize environmental exposure of exposed populations to toxigenic strains. The Agency has concluded that non-occupational and residential exposure is not likely to be greater than that which exists to naturally-occurring *A. flavus* fungal strains.

5. Drinking Water Exposure and Risk Characterization

Exposure to AF36 via drinking water is not likely to be greater than current/existing exposures. Potential risks via exposure to drinking water or runoff are adequately mitigated by, among other things, percolation through soil. Thus, exposure from the proposed use of this non-aflatoxin-producing strain of *Aspergillus flavus* AF36 is not likely to pose any incremental risk via drinking water to adult humans, infants and children. Rather, displacement of the toxigenic strains by AF36 is likely to decrease exposure and risk to the toxigenic strains of *A. flavus* in the environment and in water.

6. Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children

This microbial pesticide is intended for use on cotton, which itself is not a dietary commodity. The microbe was isolated from cottonseed and could be expected to be found there after treatment. However, AF36 is not expected to survive the heating and pressure associated with the processing of cottonseed. Residues of AF36, the microbe, will not separate into the edible fraction, cottonseed oil, thus minimizing the potential for dietary exposure. Moreover, starter cultures of AF36 are screened by thin layer chromatography and scanning fluorescence densitometry for lack of aflatoxin, according to studies submitted to the Agency. Finally, the levels of aflatoxin in cotton and its byproducts, cottonseed oil and cottonseed meal, are regulated by the FDA. Based on the submitted studies, the End-use Product, *Aspergillus flavus* AF36, demonstrates low acute oral toxicity category IV potential [BPPD Review - April 23, 1996], and category III for inhalation effects [BPPD Review -

April 2, 2003c]. The Agency has decided that the acute and chronic risks posed by dietary exposure to the pesticide via use on cotton are likely to be minimal to non-existent.

7. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

Dermal

Non-occupational dermal exposure and risk are likely to be minimal to non-existent based on:

- (i) the potential use sites, which are commercial and agricultural;
- (ii) the granular nature of the pesticide which minimizes pesticide drift;
- (iii) the low application rates;
- (iv) the methods of application of the pesticide, with no cultivation immediately after treatment and return of levels to background shortly after germination; and
- (v) the lack of reported hypersensitivity incidents.

Occupational dermal exposure to AF36 has been previously discussed and appropriate measures, such as PPE and Restricted-Entry Intervals, are required to mitigate any potential occupational dermal exposure and risk (see **Section III.4.a**).

Oral

Oral exposure would occur primarily from eating treated produce. Cotton itself is not a food commodity and, therefore, exposure via eating commodities treated with AF36 is not expected. The microbe (AF36) was isolated from cottonseed and could be expected to be found there after treatment. However, AF36 is not expected to survive the heating and pressure associated with the processing of cottonseed and partitioning of residues of AF36 or its metabolites into cottonseed oil is not likely based on the extraction method. Neither the pesticide nor its metabolites partition into the solvent or with the oil during processing and extraction. Cottonseed meal, to be used as feed for dairy and beef cattle, must meet the requirements of the Food and Drug administration. [For more discussion, see **Section III.B.3**]. Thus potential transfer of residues to meat and milk is actively monitored and mitigated in order not to exceed regulatory levels. Hence, dietary exposure to AF36, via treatment of cotton with AF36, is not expected to exceed normal background levels associated with *A. flavus* fungal strains.

Inhalation

Non-occupational inhalation exposure is likely to be minimal. The ubiquitous distribution of *A. flavus* in the environment implies that inhalation exposure to AF36 is not likely to pose an incremental risk above that which occurs during normal exposure to *A. flavus* strains. The greatest occupational inhalation exposure would occur to mixer/loaders, applicators, flaggers, markers and early entry workers. Based on the Toxicity Category III classification of the pesticide for acute inhalation effects [BPPD Review - April 02, 2003],

inhalation exposure is not likely to pose an undue risk to workers. Nevertheless, the Agency has decided that all occupationally exposed workers must wear a dust/mist filtering respirator with the NIOSH prefix N-95, P-95 or R-95, because of the microbial nature of the active ingredient.

In summary, the potential aggregate exposure, derived from (a) dietary exposure from the treated food/feed commodity, cotton, and from drinking water potentially exposed secondary to AF36 treatments of cotton, and (b) dermal and inhalation non-occupational and occupational exposure of populations exposed to AF36, is not expected or should be adequately mitigated, as long as the pesticide is used as labeled.

8. Cumulative Effects

Section 408(b)(2)(D)(v) of the FFDCFA requires the Agency to consider the cumulative effect of exposure to *Aspergillus flavus* AF36 and to other substances that have a common mechanism of toxicity. These considerations include the possible cumulative effects of such residues on infants and children. *Aspergillus flavus* AF36 does not appear to be toxic or pathogenic in test mammalian systems. Thus, there is no indication that the fungus we consider here shares any common mechanisms of toxicity with other substances. There are no other registered products containing *Aspergillus flavus* AF36 and other *A. flavus* strains abundant in the environment. The displacement of the toxigenic strain of *A. flavus* by AF36 may reduce aflatoxin contamination of cottonseed. Based on the foregoing, no cumulative or incremental effect is expected from the use of this pesticide on cotton.

9. Determination of Safety for U.S. Population, Infants and Children

There is reasonable certainty that no harm will result from aggregate exposures to residues of *A. flavus* AF36, in its use as an antifungal agent, to the U. S. population, including infants and children. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. As discussed previously, there appears to be no potential for incremental exposure and risk from this fungus in its use as an antifungal agent, since submitted studies demonstrate that the organism is not toxic in mammalian systems. The Agency has arrived at this conclusion based on the very low levels of mammalian toxicity for acute oral and pulmonary effects, with no toxicity or infectivity at the doses tested (see **Section III.B.2**). Moreover, non-occupational inhalation or dermal exposure is not expected above background levels (see **Section III.B.7**).

FFDCFA section 408(b)(2)(C) provides that EPA shall apply an additional ten-fold margin of exposure for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database unless EPA determines that a different margin of exposure will be safe for infants and children. Margins of exposure

are often referred to as uncertainty factors. In this instance, based on all the available information, the Agency concludes that the fungus, *A. flavus* AF36, is non-toxic to mammals, including infants and children. Because there are no threshold effects of concern to infants, children, and adults when *A. flavus* AF36 is used as labeled, the provision requiring an additional margin of safety does not apply. As a result, EPA has not used a margin of exposure approach to assess the safety of *A. flavus* AF36.

C. Environmental Assessment

1. Ecological Effects Hazard Assessment

Below is a summary of the ecological effects database evaluated in support of this action. The database for studies and information of toxicity of AF36 to non-target organisms are sufficient to allow conditional registration as a microbial pesticide for use on cotton.

a. Toxicity to Terrestrial Animals

(i) Avian injection (MRID 45798102, OPPTS 885.4100; Gdln 154 -17)

Certain *Aspergillus* fungal strains could be considered as infrequent or occasional pathogens in aspergillosis-related respiratory afflictions in birds. Invasive aspergillosis, which often causes mortality when it occurs, is most commonly linked to the microbial pathogen, *Aspergillus fumigatus*. Other thermotolerant *A. flavii* may also be associated with the infections. In addition, it was not clear whether AF36 is more or less pathogenic than other strains of *A. flavus*.

The registrant provided data to demonstrate that indigenous *A. flavus* is present on both cotton and wheat. Naturally occurring levels of colonization by *A. flavus* on wheat seed ranged from 0 to 100% compared to 0.5% observed in a control area without substantial levels of *A. flavus*. However, because cotton is cultivated on more acres than wheat in AZ, inhalation exposure to birds on the wheat matrix on which AF36 is grown is likely to be higher than expected.

Since the effects of AF36 had not been demonstrated in avian pulmonary systems, the Agency denied the registrant's request to waive data for this guideline requirement [No MRID: BPPD Review of Additional Rationale for Data Waiver Request, dated June 23, 1999b, (hereinafter referred to as "BPPD Review - June 23, 1999b")]. Therefore, intratracheal injection studies on the bobwhite quail were required in order to determine the infectivity/pathogenicity effects of AF36 on avian pulmonary systems. The registrant conducted these studies during the experimental use permit phase.

The potential toxicity of AF36 to young bobwhite quail (26 day old) was assessed in a maximum hazard dose avian injection study. Thirty birds received daily doses of AF36 by intratracheal instillation for five days. Observations for 30 days post-dosing showed no

clinical signs of toxicity and no treatment-related effects were evident in body weight change or food consumption. No abnormalities were observed during macroscopic *post mortem* evaluations. Bobwhite quail treated with AF36 at a mean daily inhalation dose of 1.44×10^5 cfu per bird for five consecutive days exhibited no toxic or pathogenic effects [MRID 45798102; BPPD Data Evaluation Record, dated April 16, 2003, (hereinafter referred to as "BPPD DER - April 16, 2003")]. This study was considered acceptable and used as the basis to waive the avian oral study. Because no pathogenic effects were observed in this study, no additional testing at higher Tiers was required.

(ii) Wild Mammal Testing: Acute Toxicity/Pathogenicity (MRIDs 43763405, 45307201, 45307202, 43972403, 45798201; OPPTS 885.4150; gdl. 154A-18)

Wild mammal studies can be addressed by acute oral toxicity and pulmonary infectivity/toxicity mammalian studies for health effects (see **Table 1 and Acute Oral and Acute Pulmonary studies, Health Effects**). The acute oral LD_{50} is greater than 5000 mg/kg rat body weight as demonstrated in the mammalian studies submitted for the health effects guideline requirements. No clinical signs were observed during the study, nor were abnormalities noted upon necropsy [BPPD Review - April 23, 1996]. Acute pulmonary toxicity tests in rats were conducted with intratracheal administration of $1.93 - 2.90 \times 10^8$ cfu/rat of the pesticide without Tween 80. These tests demonstrated observable clearance patterns and the active ingredient was considered neither infective nor pathogenic by the pulmonary route [BPPD Review - April 02, 2003b]. On the basis of mammalian studies, the pesticide was classified as Toxicity Categories IV and III, respectively, for acute oral toxicity and inhalation effects.

The pesticide is to be applied only to agricultural sites (cotton fields in AZ and TX). Potential exposure of wild mammals and other terrestrial animals of concern on agricultural sites is expected to be minimal. Based on the moderate to low mammalian toxicity/pathogenicity observed effects, the Agency has decided that the use of this microbial pesticide is not likely to pose incremental hazards to wild mammals, if it is used as labeled. No additional testing at higher tiers is ordinarily required, since no pathogenic effects were observed in the mammalian studies.

**(iii) Beneficial Insects
Honeybee Testing (MRID 45739102; OPPTS 885.4380; Gdl. 154-24)**

While data requirements for most non-target insects were waived (see discussion below), the Agency required that the registrant submit data to demonstrate the toxicity/pathogenicity effects of AF36 on the beneficial insect, the honey bee. A guideline study, provided to demonstrate the toxicity/pathogenicity effects of the pesticide on honey bees, was considered **Acceptable**. The exposure and potential hazard of AF36 colonized-wheat seed to foraging honey bees (*Apis mellifera* L.) on blooming cotton was assessed for 30 days, following an aerial application at label rates. On the basis of this study, AF36

applied once at 10 lbs EP/acre is not considered hazardous to honey bees [MRID 45739102; BPPD Data Evaluation Record from Alan H. Reynolds, dated April 29, 2003, (hereinafter referred to as “BPPD DER- April 29, 2003”)].

Table 3a: Eco-Toxicology Summary/Studies Evaluated

Guideline No.	Study	Status, Classification & Comments	MRID Nos.
154-17 *885.4100	Avian injection	No incremental hazards of AF36 for avian species are anticipated for this use. Young bobwhite quail treated with <i>Aspergillus flavus</i> AF36 at a mean daily inhalation dose of 1.44×10^5 cfu per bird for five consecutive days exhibited no toxic or pathogenic effects during the 30 day observation period.	45798102 45307202
154-18 *885.4150	Wild mammal testing	No incremental hazards of AF36 for wild mammalian species are anticipated for this use. The mammalian acute oral pathogenicity and acute pulmonary toxicity tests (OPPTS 885.3050 and 885.3150), support this finding.	43763405 45307201 45307202 43972403
154-24 *885.4380 *850.3040	Honey bee testing, Tier 1 Field Testing of Pollinators	No incremental hazards of AF36 for honeybees are anticipated for this use. The exposure and potential hazard of <i>Aspergillus flavus</i> AF36 colonized-wheat seed to foraging honey bees (<i>Apis mellifera</i> L.) on blooming cotton was assessed for 30 days, following an aerial application at label rates. <i>Aspergillus flavus</i> AF36 applied once at 10 lbs/acre was not hazardous to honey bees.	45739102

*885 series = OPPTS Microbial Pesticide Test Guideline Numbers.

b. Data Waivers: Ecological Effects

The following ecological effects studies were waived:

- (i) Avian oral toxicity/pathogenicity [**MRID 44464202; OPPTS 885.4050; Gdln 154-16**]
- (ii) Freshwater Fish testing (**OPPTS 885.4200; Gdln 154-19**)
- (iii) Freshwater Aquatic Invertebrate Testing (**OPPTS 885.4240; Gdln 154-20**)
- (iv) Estuarine and Marine Animal testing (**OPPTS 885.4280; Gdln 154-21**)
- (v) Non-target Plant studies (**OPPTS 885.4300; Gdln 154-22**)
- (vi) Non-target Insect testing (**OPPTS 885.4340; Gdln 154-23**)

Justifications for data waivers

Rationales for these data waiver requests are summarized below:

(i) Avian Species: Toxicity/Pathogenicity

Avian oral toxicity/pathogenicity [MRID 44464202; OPPTS 885.4050; Gdln 154-16]

A request to waive test data for avian oral infectivity/pathogenicity studies was submitted, as well as surrogate data demonstrating the effects of another pesticide on birds in cotton fields. The latter claimed that the likelihood of adverse impacts was considered to be low to very low, based on the limited use of cotton fields by birds and existing agricultural practices which interfere with nesting. Risk was defined as impact on survival or reproduction. Bird census data were reported from cotton fields and surrounding environments in Arizona, Texas, and Alabama/Mississippi. Other aspects of the study concluded that the primary activities of birds in cotton fields in AZ were perching (30%) followed by foraging (23%). The registrant also argued that birds are more likely to occupy wheat than cotton fields at the time of application of AF36, and that wheat seeds already are populated with the naturally occurring *Aspergillus flavus* strains.

Agency review of the request to waive avian oral studies concluded that a number of native and endangered avian species may be present in AZ cotton fields at the time of application of AF36 treated wheat seeds [MRID 44464202; BPPD Review - Simulated and Actual Field Testing, dated June 23, 1999a (hereinafter referred to as "BPPD Review - June 23, 1999a"). However, desert habitats, especially riparian areas, are more likely to provide better bird habitats than cotton fields. In addition, because insect herbivory is heavily managed in cotton fields, they provide a poor food source for insectivorous or omnivorous birds [MRID 44464202; BPPD Review - June 23, 1999a]. Furthermore, while carnivorous birds of prey (see **Endangered Species**) also are likely to be exposed, their primary food source would not be wheat or cotton seeds. As a result, no exposure or risks are anticipated to the carnivorous and omnivorous endangered avian species.

Red-winged blackbirds were the most commonly observed avian species, comprising about 70 to 80 percent of the bird population in the cotton agroecosystem. Their stomach content revealed a diet consisting of a variety of invertebrates and vegetation. According to the surrogate study submitted by the registrant, birds were not often observed foraging, but this probably is due to the difficulty of observing bird behavior after the cotton canopy grew. However, the surrogate study did advance that birds do not eat cotton seeds. Nonetheless, it was unclear whether birds would eat the wheat seeds which are the matrix on which AF36 grows.

However, while the oral route may be a source of exposure, the main route of exposure for aspergillosis is pulmonary [MRID 43763403: BPPD Review from Gail Tomimatsu and Robert I. Rose, dated April 24, 1996, (hereinafter referred to as "BPPD

review - April 24, 1996"). The possibility was also considered that the incremental avian dietary exposure and risk may not be greater than that which occurs normally, because of the natural abundance of *Aspergillus* fungal strains in the areas to be treated. Since only 1000 to 22000 acres of cotton were to be treated during the EUP, the Agency decided to waive data for avian oral exposure during the experimental phase. Because inhalation was the major route of exposure for aspergillosis, Tier 1 avian injection data were required, and were conducted during the EUP. Based on the low toxicity potential in the avian injection study, discussed above, the avian oral study was waived for this conditional registration.

**(ii - iv) Aquatic Animals- Freshwater and Estuarine
(Gdlns. 154-19, 154-20, 154-21)**

Exposure of freshwater vertebrates and invertebrates to *Aspergillus flavus* AF36 is considered likely if cotton fields are adjacent to a freshwater source. However, in consideration of the natural population fluctuations of *A. flavus*, the intended use pattern, and data from soil and air population monitoring [MRID #s 45307201, 45307202; BPPD Review, May 15, 2003], such incremental exposures of AF36 would not present a hazard to aquatic organisms.

Estuarine and marine vertebrates and invertebrates are less likely to be exposed to AF36 than their freshwater relatives. In addition to the indigenous presence of AF36 in the ecosystem, the waiver rationale claimed: a) that minimal exposures and runoff or drift to aquatic ecosystems will occur because of directed application to cotton soils with a granular formulation; and b) no reports of *A. flavus* pathogenesises to aquatic organisms. The Agency considered this rationale acceptable. Accordingly, all toxicity/pathogenicity studies for aquatic organisms are waived for freshwater fish (OPPTS 885.4200), freshwater aquatic invertebrates (OPPTS 885.4240), and estuarine and marine vertebrates and invertebrates (OPPTS 885.4280) for this particular application of AF36 to cotton in AZ and TX.

(iv) Non-target Plant studies (OPPTS 885.4300; Gdln. 154-22)

Results of soil and air monitoring studies showed that a single, seasonal, prebloom application of AF36 does not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations [MRID#s 45307201, 45307202; BPPD Review, May 15, 2003]. Although the Agency waived pathogenicity testing to non-target plants [BPPD Review, April 24, 1996; BPPD Review, June 23, 1999], the applicant also formally submitted the rationale which asserts that AF36 is a naturally occurring strain of *Aspergillus flavus*, a ubiquitous saprophyte commonly found in soil and plant tissues. Therefore, and for these reasons, this guideline requirement was waived.

(v) Non-target Insect testing (OPPTS 885.4340; Gdln. 154-23)

Results of soil and air monitoring studies showed that a single, seasonal, prebloom application of AF36 does not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations [MRID#s 45307201, 45307202; BPPD Review, May 15, 2003]. Also, the exposure and potential hazard of *A. flavus*-colonized wheat seed to foraging honey bees (*Apis mellifera* L.) on blooming cotton was assessed for 30 days, following an aerial application at label rates. *Aspergillus flavus* AF36 applied once at 10 lbs/acre was not hazardous to honey bees [MRID 45739102; BPPD Data Evaluation Record, dated April 29, 2003]. Therefore, no incremental hazards of AF36 are anticipated for resident non-target insects.

The Agency waived pathogenicity testing to most non-target insects [MRIDs 43763403, 43763405; BPPD Review, June 23, 1999], and requested that the applicants formally submit a rationale to waive pathogenicity testing to insects, except honeybees. The acceptable rationale asserts that AF36 is a naturally occurring strain of *Aspergillus flavus*, a ubiquitous saprophyte commonly found in soil and plant tissues, and that actual field use under an EUP resulted in no reports of adverse effects to insects. Soil and air population studies for AF36 and *Aspergillus*, honeybee field tests (OPPTS 885.4380 and 850.3040), and acceptable waiver rationale support this finding.

Table 3b: Eco-Toxicology Summary: Data Waivers

Guideline No.	Study	Status, Classification & Comments	MRID Nos. Reviewed
154-16 *885.4050	Avian Oral Toxicity	No incremental hazards of AF36 for avian species are anticipated for this use. Results of soil and air population studies, the avian injection test (OPPTS 885.4100), and acceptable waiver rationale support this finding.	43763403 43763405 44464202 44452615 45739103 45307201 45307202 45798102
154-19 *885.4200	Fresh water fish testing	No incremental exposures of AF36 for freshwater aquatic invertebrates are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> and acceptable waiver rationale support this finding.	43763403 43763405 45307201 45307202 Rationale for waiver acceptable
154-20 *885.4240	Fresh water aquatic invertebrate testing		
154-20 *885.4280	Estuarine and marine animal testing		
154-22 *885.4300	Non-target plant studies, Tier 1	<i>A. flavus</i> strains are naturally abundant in plant debris and soil. No significant exposure above background levels expected.	Rationale for waiver acceptable.
154-23 *885.4340	Non-target insect studies	No incremental exposures of AF36 for insects are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> , honeybee field tests (OPPTS 885.4380 and 850.3040) and acceptable waiver rationale support this finding.	43763403 43763405

*885 series = OPPTS Microbial Pesticide Test Guideline Numbers.

2. Environmental Assessment

Environmental Assessment

Data indicate that populations of *A. flavus* fluctuate throughout the year. More importantly, these data indicate that shifts in population numbers do not appear to be

associated with application of AF36. Results of multiple year soil and air population monitoring studies indicate that the number of *A. flavus* conidia increase within a few days of application as is expected of the germinating microbial pesticide. The results also suggest that AF36 applications do not significantly increase the overall quantity of *Aspergillus flavus* at cotton crop maturity, nor in the soil one year after application. These data suggest that the pesticidal mode of action of AF36 may be attributed to competitive displacement of the aflatoxin-producing strains of *A. flavus*.

3. Ecological Exposure and Environmental Expression Risk Characterization

It is anticipated that a single, seasonal, prebloom application of AF36 should not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations. Incremental exposures of AF36 to the environment and to non-target organisms which inhabit or pass through the treated cotton agroecosystems do not present an adverse concern as a consequence of this proposed use of AF36. The ecological test and environmental expression data support a conclusion of reasonable certainty that no incremental hazards to non-target organisms or to the environment are anticipated as a result of the intended use of AF36 on cotton [BPPD Review - May 15, 2003].

No further testing for ecological effects or environmental expression is necessary for AF36. However, for environmental expression in other cotton-growing states or regions, e.g., Texas, additional testing or research is required to satisfy concerns for product performance, or efficacy in reducing aflatoxin levels in cottonseed.

D. Efficacy Data

PR Notice 2002-1 lists aflatoxin as a public health hazard, for which product performance or efficacy data are required according to 40CFR 158.202(i). To demonstrate that this pesticide may reduce aflatoxin-producing strains and does not significantly increase *A. flavus* populations above background levels, the applicant provided product performance or efficacy data from multiple years of soil and air monitoring studies.

Aflatoxin, one of the most potent human carcinogens, is the metabolite of concern produced by the target pest, aflatoxin-producing strains of *Aspergillus flavus*. As such, the Agency considers aflatoxin a public health hazard. Few alternatives, if any, exist to displace aflatoxin-producing *A. flavus* strains from cotton and other crops. In the soils of cotton-producing areas of AZ and south TX, especially in the dry regions, the toxigenic strains are prominent. Decontamination of crops via ammoniation is costly, not available universally and decreases the value of the crop. Other methods to reduce aflatoxin formation include manipulation of harvest date, costly irrigation practices, and different methods of harvesting and storage practices.

Efficacy data submitted to the Agency include monitoring of soil and air levels of the toxigenic and non-aflatoxin-producing strains of *A. flavus* in the field and on the crops. Results from the environmental expression and population monitoring studies during the experimental program demonstrate that a single, seasonal, prebloom application of AF36 on cotton fields may incite significant changes in the incidence of toxigenic *A. flavus* strains resident in the agroecosystem, without altering the overall quantity of *A. flavus*. Soil and air population counts of *A. flavus* from treated fields were associated with concomitant decreases in incidences of toxigenic *A. flavus*, for many of the treated areas [MRIDs 45307201, 45307202: BPPD review - May 15, 2003]. Reducing the aflatoxin-producing populations of fungi, and the concomitant reduction of aflatoxin, a potent carcinogen, is in the public interest.

IV. PUBLIC INTEREST FINDING

The Agency believes the use of AF36 under this conditional registration would be in the public interest. The criteria for Agency evaluation of public interest findings are outlined in 51 FR No. 43, Wednesday March 5, 1986. Under part IV.A, the proposed product may qualify for an automatic presumptive finding that the proposed conditional registration is in the public interest if it is for a minor use, is a unique replacement for pesticides of concern, or is for use against a public health pest.

Aflatoxin, a potent human carcinogen that is considered a public health hazard by the Agency, is the metabolite of concern produced by the target pest, aflatoxin-producing strains of *A. flavus*. There is no pesticide registered to displace aflatoxin-producing strains of *A. flavus*. The pesticide product, containing *A. flavus* AF36, is proposed to displace toxic strains of *A. flavus* on cotton in AZ and TX. Since 1996, AF36 has been used in these 2 states in an experimental program by the USDA Agricultural Research Service and the Arizona Cotton Research and Protection Council. No adverse effects have been reported during this experimental phase. Even though cotton itself is not a minor crop, the proposed use is regional for AZ and TX, which represents less than 25 percent of total US cotton to be treated. Based on these rationales, the Agency has determined that 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 byproducts, and is in the public interest.

V. RISK MANAGEMENT AND REGISTRATION DECISION

A. Determination of Eligibility

Section 3(c)(7)(C) of FIFRA provides for the conditional registration of a pesticide containing a new active ingredient (*i.e.*, not contained in any currently registered pesticide) “for a period reasonably sufficient for the generation and submission of required data . . . on the condition that by the end of such period the Administrator receives such data and the data do not meet or exceed risk criteria” identified in regulations issued under FIFRA “and on such other conditions as the Administrator may prescribe.” Such a conditional registration will be granted “only if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest.”

Aspergillus flavus AF36 is eligible for a conditional registration because its proposed use on cotton in AZ and TX is in the public interest, and AF36 is not likely to pose any unreasonable risk to health or the environment as discussed in this document. Certain conditions apply to this eligibility and the applicant must take certain actions (*e.g.*, generate and provide certain data) within the time frames outlined in **Section VI** of this document.

B. Regulatory Position

1. Conditional/Unconditional Registration Eligible use

Data submitted are sufficient for a conditional registration of *Aspergillus flavus* AF36 for use on cotton in Arizona and Texas in accordance with its label directions. While the registrant has provided demonstrable reduction of aflatoxin-producing *A. flavus* in AZ during the EUP, similar efficacy studies have not been performed in TX. The registrant has provided data from a small scale trial in TX and sought to bridge the Arizona data to TX. However, the areas tested in TX were small and may not accurately reflect the proliferation of AF36 which facilitates competitive displacement of the aflatoxin-producing strains. As a condition of registration, the Agency requires efficacy studies from large scale trials to confirm the bridging of data from AZ to TX.

2. Tolerance Reassessment

The exemption from temporary tolerance was reassessed to comply with FQPA during the extension of the Experimental Use Permit to allow an exemption from temporary tolerance of *Aspergillus flavus* AF36 on cotton in AZ (FR. May 26, 1999. (Vol. 64, No. 101) [Page 28371-28374]; FR: June 30, 1999 (Vol. 64, No. 125))[Page 35049-35051]. Additional

mammalian pulmonary infectivity/toxicity effects data were provided in 2002 to support the filing of the pesticide petition affiliated with this proposed Section 3(c) registration. The current database supports a reassessment of the temporary exemption from tolerance which complies with the requirements of FQPA. A final rule to revise 40 CFR §180.1206 to include a permanent exemption from the requirement of a tolerance for residues of *Aspergillus flavus* AF36 will be published in the Federal Register concurrent with this Section 3(c)(7)(C) conditional registration.

3. Ineligible Uses

This document summarizes the database supporting the eligibility of *Aspergillus flavus* AF36 for a conditional registration for use on cotton in AZ and TX only. Any other application of this pesticide, not in compliance with Agency requirements, will constitute a misuse.

4. CODEX Harmonization

There are no Codex harmonization considerations since there is no Codex Maximum Residue Limits set for food use of this active ingredient.

5. Non-food Re/Registrations

This is a new active ingredient and, therefore, not the subject of reregistration at this time.

6. Risk Mitigation

There is minimal or negligible potential risk to non-target organisms (plants and wildlife), and to ground and surface water contamination through the proposed use of products containing *Aspergillus flavus* AF36 as discussed in this document. No mitigation measures required at this time for dietary risk, including risk due to exposure via drinking water. Appropriate PPE is required for pesticide handlers. These include long sleeve shirt, long pants, shoes and socks, goggles, and a dust/mist filtering respirator with the NIOSH prefix N-95, R-95, P-95. In addition to this gear, early entry postapplication workers, must wear coveralls during the Restricted-entry Interval (REI) of 4 hours (*see **Occupational Exposure and Risk***). The pesticide is to be applied to cotton fields in Arizona and Texas only. The product label will also bear Environmental Hazards text to mitigate any potential risk as determined by reviewed data and use sites.

7. Endangered Species Statement

Currently, the Agency is developing a program (The Endangered Species Protection Program) to identify all pesticides whose use may cause potential adverse impacts on endangered and threatened species and their habitats. To aid in the identification of threatened and endangered species and their habitats, several companies have formed an Endangered Species Task Force (EST) under the direction of the American Crop Protection Association (ACPA). Moreover, the EST will assist in providing species location information at the subcounty level, and, particularly, if an endangered species occurs in areas where pesticides would be used. This information will be useful once the Endangered Species Protection Program has been implemented.

The Agency reviewed avian endangered species data in connection with the EUP for this active ingredient, as discussed under the section regarding avian guideline requirements. Even though some avian endangered species are reported in AZ, none of them were reported in or around cotton fields (MRIDs 444642-02, 444526-15). These birds may not have been observed by census takers, most likely because they are rare. Increased exposure above background levels of *A. flavus* is not expected based on the feeding habits and preferred habitats of some of these species. For example, birds of prey do not feed on wheat seeds and are, therefore, not expected to receive increased exposure from AF36.

The Masked Bobwhite quail could be expected to feed on AF36-treated wheat seeds if this bird is found in cotton fields. However, the Masked Bobwhite currently survives only on reserves, where it is protected from predation from coyotes. These birds are not expected to survive outside the reserves, which are several miles away from the cotton fields [BPPD DER - April 24, 1996]. Pending acceptable data about the effects of AF36 on avian species, the Agency required labeling to protect certain plovers and other endangered species in Texas during the EUP.

Information later submitted to the Agency indicates that certain insectivorous/invertebrate-feeding plovers do not feed on the wheat seed, and are not found in the cotton agriculture/agroecosystem habitat. Furthermore, inhalation, rather than oral exposure is associated with aspergillosis, which may be caused by certain *Aspergillus* fungal strains such as *fumigatus*. Intratracheal instillation of AF36 in bobwhite quail demonstrated no toxic or pathogenic effects (see **Section IIIC. Ecological Effects: Avian injection**).

No incremental hazards of AF36 are anticipated to endangered mammals on the basis of results from acute oral and acute pulmonary toxicity tests in mammalian systems [BPPD review - April 24, 1996; [BPPD review - April 02, 2003c].

The Agency has made a no effect finding for the use of pattern of AF36. Thus, no labeling is required for endangered species at this time.

C. LABELING RATIONALE

It is the Agency's position that the labeling for manufacturing products containing *Aspergillus flavus* strain AF36 must comply with the pesticide labeling requirements in existence when such products are registered.

1. Manufacturing Use Product Labeling

The label must include appropriate statements to indicate that the registered product is a manufacturing use product (MUP) if the intent is to use the product to formulate into end-use products (EP). Long sleeved shirt, long pants, shoes, socks, goggles, gloves and a dust/mist filtering respirator with the NIOSH prefix N-95, P-95 or R-95 are required when handling or formulating the MUP into the EP.

The following NPDES statement must be placed on the manufacturing use product for the active ingredient, *Aspergillus flavus* strain AF36, at this time.

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA."

2. End-use Product Labeling

It is the Agency's position that the labeling for End-use Product products containing *Aspergillus flavus* strain AF36 must comply with the pesticide labeling requirements in existence when such products are registered.

a. Human Health Hazard (i) Worker Protection Standard

Any product whose labeling reasonably permits use in the production of an agricultural plant on any farm, forest, nursery, or greenhouse must comply with PR Notice 93-7, "Labeling Revisions required by the Worker Protection Standard (WPS), and PR Notice 93-11, "Supplemental Guidance for PR Notice 93-7", which reflect the WPS (40 CFR part 156, subpart K). These labeling revisions are necessary to implement the Worker Protection Standard for Agricultural Pesticides (40 CFR part 170). Unless otherwise specifically

directed, all statements required by PR Notices 93-7 and 93-11 are to be on the product label exactly as instructed in those Notices.

The labels and labeling of all products must comply with EPA's current regulations and requirements as specified in 40 CFR 156.10 and other applicable notices, such as, and including the WPS labeling.

Workers and handlers (including mixer/loader, applicators) applying this product must wear long sleeved shirt, long pants, shoes, socks, goggles and gloves, as well as a dust/mist filtering respirator with NIOSH approval number prefix -95, R-95 or P-95. Postapplication agricultural workers and early-entry workers must wear coveralls in addition to the PPE above when entering treated areas during the restricted entry interval (REI) of 4 hours.

(ii) Non-Worker Protection Standard

There are no non-WPS uses of this active ingredient.

(iii) Other Precautionary Labeling

The Agency has examined the toxicological data base for *Aspergillus flavus* strain AF36 and concluded that the precautionary labeling required during this conditional registration process (i.e. Signal Word, First Aid Statements, WPS statements for pesticide handlers, and other label statements) adequately mitigates the risks associated with the proposed uses. Additional labeling may be required for other uses of products containing *A. flavus* AF36 on a case by case basis.

b. Environmental Hazards Labeling

Standard Environmental Hazards labeling statements are required for this aerial agricultural application.

Provided the following statements are placed in the environmental hazards statement, the risk of exposure to *Aspergillus flavus* AF36 is minimal to nonexistent to non-target organisms including endangered species:

“Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of rinsate or equipment washwaters.”

3. Application Rate

It is the Agency's position that the labeling for the pesticide products containing *Aspergillus flavus* strain AF36 must comply with the current pesticide labeling requirements. The pesticide is to be applied as a granular aerial or ground application at the rate of 10 pounds colonized wheat seeds (<0.01 lb ai) per acre. Only 1 prebloom application per season is allowed.

D. LABELING

a. Manufacturing Use Product

There is no separate manufacturing use product (MP) registered at this time. However MUP labeling is required since this pesticide product is considered as produced by an integrated process.

b. End-use Product

End-use Product name: *Aspergillus flavus* AF36

Ingredient Statement:	w/w
<i>Aspergillus flavus</i> strain AF36.....	0.0008%
Inert Ingredients	99.0002%

Total 100.00 %*

* viability of End-use Product is 3000 cfu/g

Based on the evaluation of the acute oral and acute pulmonary toxicity/infectivity studies submitted in support of the conditional registration of the product, containing *Aspergillus flavus* strain AF36, the signal word is "CAUTION". Signal words for other end-use products containing this active ingredient will vary depending on the toxicity/pathogenicity evaluations of those products.

VI. ACTIONS REQUIRED BY REGISTRANTS

Reports of incidents of adverse effects to humans or domestic animals are required under FIFRA, Section 6(a)(2) and incidents of hypersensitivity under 40 CFR Part 158.690(c), guideline reference number 152-16. There are no data requirements, label changes and other responses necessary for the reregistration of the end-use product since the product is being registered after November 1984 and is, therefore, not subject to reregistration. For the same reason, there are also no existing stocks provisions at this time. Before releasing these products for shipment, the registrant is required to provide appropriate labels and other Agency requirements as discussed in this BRAD. The applicant must provide the following data within 30 months of the conditional registration date as shown below in Table 4.

1. Guidelines 151-10 through 151-16 (OPPTS gdln 885.1300): Product Identity, Manufacturing Process and Quality Control

Analyses of 5 batches are required at production and must include data relevant to detection, identification, enumeration and rejection limits of metabolites (including aflatoxin) and potential human pathogens (bacterial and fungal), using quality control and assurance methods to be used during large scale production. Batch analysis must also include:

- (i) certifications of limits;
- (ii) identification of *A. flavus* AF36 by either DNA analysis or some other method different from the vegetative compatibility method now in use;
- (iii) analysis and quantification of metabolites and other unintentional ingredients;
- (iv) identification and enumeration of potential human pathogens;
- (v) storage stability; and
- (vi) viability data.

All batches containing metabolites or unintentional ingredients of toxicological concern, or human pathogens above regulatory levels must be destroyed. The data from production batches (i thru vi, inclusive, listed above) will be a condition of registration and must be submitted within the time frames noted in Table 4 of this BRAD (within 30 months of the date of this conditional registration action).

2. Non-guideline study: Efficacy (Product Performance)

Efficacy data are required from a large-scale field trial in TX to demonstrate that *Aspergillus flavus* AF36 reduces aflatoxin-producing strains of *Aspergillus flavus*.

Table 4: Data required

Guideline	Title of Study	Data required	Date due
*885.1300 151-12	Discussion of Formation of Unintentional Ingredients	Human pathogen and metabolite identification and quantification (including aflatoxin quantification by HPLC).	During production of 5 batches, within 30 months after conditional registration date.
*885.1400 151-13	Analysis of Samples	5 batch analysis to include another method apart from VCG analysis to identify <i>Aspergillus flavus</i> AF36, viability and storage stability data.	During production of 5 batches, within 30 months after conditional registration date.
*885.1500 151-15	Certification of limits	Standard data requirement for production batches.	During production of 5 batches, within 30 months after conditional registration date.
Non-guideline: required for public health hazard	Efficacy	Efficacy (product performance) data to demonstrate the reduction of toxigenic strains by <i>A. flavus</i> AF36 in Texas.	Within 30 months after conditional registration date.

*OPPTS Harmonized Guidelines

VII. APPENDICES

APPENDIX A - Use sites

Table 5 lists the use sites for the product. The registrant must comply with the appropriate labeling requirements before releasing products containing *Aspergillus flavus* AF36 as the active ingredient for shipment.

Table 5: Use Site Conditional registration

Prebloom application by ground or air to cotton in Arizona, Texas.	Official date registered:
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APPENDIX B - Citations Considered to be part of the Data Base Supporting the Conditional registration of *Aspergillus flavus* strain AF36.

CITATIONS/BIBLIOGRAPHY

Studies submitted in support of this registration action and Pesticide Petition 8E5001

- 43763400 USDA/ARS and IR-4 (1995) Submission of Product Chemistry, Toxicity, and Risk Data in Support of an Experimental Use Permit for *Aspergillus flavus* AF36. Transmittal of 5 Studies.
- 43763401 Cotty, P. (1995) *Aspergillus flavus* Isolate AF36--Product Identity and Disclosure of Ingredients, Manufacturing Process and Discussion on the Formation of Unintentional Ingredients: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS. 85 p.
- 43763402 Cotty, P. (1995) *Aspergillus flavus* Isolate AF36--Analysis of Samples, Certification of Ingredient Limits, Analytical Methods for Certified Limits, and Physical and Chemical Properties: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS. 8 p.
- 43763403 Cotty, P.; Hartman, C. (1995) *Aspergillus flavus* Isolate AF36--Safety Data in Support of Petition Proposing a Temporary Exemption from the Requirements of a Tolerance for *Aspergillus flavus* for Use in Cotton Production: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS and IR-4. 882 p.
- 43763404 Cotty, P. (1995) *Aspergillus flavus* Isolate AF36: Hypersensitivity Incidents with Microbial Pest Control Agents: Statement of Finding No Hypersensitivity: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS. 4 p.
- 43763405 Cotty, P.; Hartman, C. (1995) *Aspergillus flavus* Isolate AF36: Product Performance Data: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS and IR-4. 145 p.
- 43972400 Interregional Research Project No. 4 (1996) Submission of Product Analysis and Toxicology Data in Support of an Experimental Use Permit for *Aspergillus flavus* AF36. Transmittal of 3 Studies.
- 43972401 Cotty, P. (1996) *Aspergillus flavus* Isolate AF36--Analysis of Samples, Certification of Ingredient Limits, Analytical Methods for Certified Limits: Amendment No. 1 to MRID No. 43763404: Lab Project Number: PR 52B: 52B. Unpublished study prepared by Southern Regional Research Center, USDA/ARS. 6 p.

- 43972402 Cotty, P. (1996) *Aspergillus flavus* Isolate AF36: Hypersensitivity Incidents with Microbial Pest Control Agents: Statement of Finding of No Hypersensitivity: Amendment No. 1 to MRID No. 43763404: Lab Project Number: 52B: PR 52B. Unpublished study prepared by Southern Regional Research Center, USDA/ARS. 4 p.
- 43972403 Shelton, L. (1996) Acute Oral Toxicity Study in Rats: (*Aspergillus flavus* AF36): Final Report: Lab Project Number: M96AG84.6G31: MA M96AG84.6G31. Unpublished study prepared by Microbiological Associates, Inc. 59 p.
- 43990000 Interregional Research Project No. 4 (1996) Submission of Product Chemistry Data in Support of the Application for Experimental Use Permit for *Aspergillus flavus* AF36. Transmittal of 1 Study.
- 43990001 Cotty, P. (1996) *Aspergillus flavus* Isolate AF36--Product Identity and Disclosure of Ingredients, Manufacturing Process, and Discussion on the Formation of Unintentional Ingredients: Amendment No. 1 to MRID 43763401: Lab Project Number: PR 52B. Unpublished study prepared by USDA/ARS, Southern Regional Research Center. 6 p.
- 44597000 Interregional Research Project No.4 (1998) Submission of Product Chemistry Data in Support of the Petition for Tolerance of *Aspergillus flavus* isolate AF36 in/on Wheat. Transmittal of 1 Study.
- 44597001 Cotty, P.; Antilla, L. (1998) *Aspergillus flavus* Isolate AF36 Manufacturing Process and Discussion on the Formation of Unintentional Ingredients. Amendment No. 2 MRID 43763401: Lab Project Number: 52B. Unpublished study prepared by USDA/ARS, Arizona Cotton Research and Protection Council and Rutgers Univ. 38 p.
- 44626100 Interregional Research Project No. 4 (1998) Submission of Product Chemistry Data in Support of the Petition for Tolerance of *Aspergillus flavus* isolate AF36 in/on Cotton. Transmittal of 1 Study.
- 44626101 Cotty, P.; Antilla, L. (1998) *Aspergillus flavus* isolate AF36-Analysis of Samples, Certification of Ingredient Limits, Analytical Methods for Certified Limits: Amendment No. 2 to MRID No. 43763402: Lab Project Number: 52B. Unpublished study prepared by USDA/ARS, and Arizona Cotton Research and Protection Council. 33 p.

- 44713700 Interregional Research Project No.4 (1998) Submission of Product Chemistry Data in Support of the Petition for Tolerances of *Aspergillus flavus* in/on Cotton. Transmittal of 1 Study.
- 44713701 Cotty, P.; Antilla, L. (1998) *Aspergillus flavus* isolate AF36--Amended Manufacturing Process--Amendment No.3: Lab Project Number: 52B. Unpublished study prepared by IR-4. 21 p.
- 45307200 USDA/ARS Southern Regional Research Center (2001) Submission of Environmental Fate Data in Support of the Petition for Tolerance of *Aspergillus flavus* Isolate AF36/Cotton in/on Cotton. Transmittal of 2 Studies.
- 45307201 Cotty, P. (2001) *Aspergillus flavus* Isolate AF36: Safety Information (Soil and Air Monitoring of Populations of *A. flavus*): Lab Project Number: 52B. Unpublished study prepared by Interregional Research Project No.4. 130 p.
- 45739100 Interregional Research Project No. 4 (2002) Submission of Toxicity and Exposure Data in Support of the Petition for Tolerance of *Aspergillus flavus* on Cotton. Transmittal of 4 Studies.
- 45739103 Smith, D.; Cotty, P.; Braverman, M.; et al. (2002) *Aspergillus flavus* Isolate AF36: Non-Target Organism and Environmental Safety Information: Lab Project Number: IR-4 PR NO.52B: Unpublished study prepared by Soil & Crop Sciences, Southern Regional Research Center USDA/ARS, Rutgers University and Arizona Cotton Research and Protection Council. 57 p.
- 45739101 Blanchard, E.; Carter, J. (2002) *Aspergillus flavus* AF36: Acute Pulmonary Toxicity and Pathogenicity to the Rat: Interim Report: Lab Project Number: UAR/006. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 86 p. {OPPTS 885.3150}
- 45739104 Antilla, L., Cotty, P.; Braverman, M. (2002) *Aspergillus flavus* Isolate AF336: Hypersensitivity Incidents: Lab Project Number: 52B. Unpublished study prepared by Arizona Cotton Research and Protection Council, Southern Regional Research Center and Rutgers University. 18 p.
- 45739102 Mayer, D. (2001) Honey Bee Field Study of *Aspergillus flavus* AF36 in Cotton: Lab Project Number: WSU 00-011. Unpublished study prepared by Washington State University. 30 p. {OPPTS 850.3040, 885.4380}
- 45307202 Cotty, P. (2001) *Aspergillus flavus* Isolate AF36 Non-target Organism and Environmental Safety Information (Soil and Air Monitoring of Populations of *A.*

flavus): Lab Project Number:52B. Unpublished study prepared by Interregional Research Project No.4. 130 p.

45739101 Blanchard, E.; Carter, J. (2002) *Aspergillus flavus* AF36: Acute Pulmonary Toxicity and Pathogenicity to the Rat: Interim Report: Lab Project Number: UAR/006. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 86 p. {OPPTS 885.3150}

Federal Register Publications

1. Federal Register: February 28, 1996 (Volume 61, Number 40)[Page 7512-7513]. *Aspergillus flavus* Isolate AF36; Notice of Filing of a Pesticide Petition
2. Federal Register: June 14, 1996 (Volume 61, Number 116)[Notices] [Page 30235-30236] *Aspergillus flavus* AF 36; Establishment of Temporary Exemption from the Requirement of a Tolerance (expiration May 29, 1999)
4. Federal Register: February 19, 1999 (Volume 64, Number 33)[Page 8358-8360] Notice of Filing of Pesticide Petitions (to amend exemption from tolerance to apply 20,000 acres, extend date to Dec. 2000]
5. Federal Register: May 26, 1999. *Aspergillus flavus* AF36. Extension of temporary exemption from tolerance and amendment to comply with the Food Quality Protection Act (FQPA) of 1996 (Vol. 64, No. 101) [Page 28371-28374]. Extend to 12/30/2000.
6. Federal Register: June 30, 1999 (Volume 64, Number 125)[Page 35049-35051]. *Aspergillus flavus* AF36; Exemption from Temporary Tolerance, Technical Amendment extend to 12/30/2001
7. Federal Register: May 23, 2001. (Volume 66, Number 100)] [Page 28383-28386] *Aspergillus flavus* AF36; Extension of Temporary Exemption From the Requirement of a Tolerance - Final Rule to expire on December 30, 2003.
8. Federal Register: March 25, 2002 (Volume 67, Number 57)[Page 13628-13630] Notice of Filing a Pesticide Petition to Amend An Existing Tolerance for a Certain Microbial Pesticide Chemical in or on Food [PP 5E4575].
9. Federal Register: July 17, 2002 (Volume 67, Number 137)[Page 46884-46888]. *Aspergillus flavus* AF36; Amendment, Temporary Exemption From the Requirement of a Tolerance expires Dec 30, 2004.
10. Federal Register: February 14, 2003, (Vol. 68, No. 31)[Page 7554-7558]. *Aspergillus flavus* AF36; Notice of Filing a Pesticide Petition to Establish an Exemption from a Tolerance for a Certain Pesticide Microbial Agent in or on Food.
11. Federal Register. March 12, 2003. *Aspergillus flavus* AF36; Notice of receipt of an application to register a new active ingredient. (Vol. 68, No. 48)[Page 11841-11843].

12. Registration of New Active Ingredient (to be published 2003).
13. Final Rule: Exemption from Tolerance (to be published 2003).

BPPD Data Evaluation Records/Reviews Health Effects

Schaffer, Cindy and Sjoblad, Roy. USEPA, OPP/BPPD. April 23, 1996. Data Evaluation Record of MRID 43972403: Acute Oral Toxicity Study in Rats.

Watson, Michael T. and Kough, John. USEPA, OPP/BPPD. March 29, 1999. BPPD Review of MRID 44626101: Analysis of Samples.

Watson, Michael T. and Kough, John. USEPA, OPP/BPPD. May 14, 1999. BPPD Review of Supplementary Information Analysis of Samples.

Etsitty, Carl, and Kough, John. USEPA, OPP. April 02, 2003a. BPPD Review of MRID 45739101: Dose for Acute Pulmonary study.

Etsitty, Carl, and Kough, John. USEPA, OPP. April 02, 2003b. BPPD Review of MRID 45798101: Acute Pulmonary Study with Tween 80.

Etsitty, Carl, and Kough, John. USEPA, OPP. April 02, 2003c. BPPD Review of MRID 45798201: Acute Pulmonary Study without Tween 80.

Etsitty, Carl, and Kough, John. USEPA, OPP. April 02, 2003d. BPPD Review of MRID 45739104: Hypersensitivity incidents.

Ecological, Environmental Effects

Tomimatsu, G. S. and Robert H. Rose. USEPA, OPP. April 24, 1996. BPPD Review of Information Submitted by USDA Southern Regional Research Center/IR-4 for an Experimental Use Permit for *Aspergillus flavus* AF36; Request for Waiver for Non-Target Plant Testing.

Tomimatsu, G. S. and Robert H. Rose. USEPA, OPP. April 24, 1996. BPPD review of MRID 43763403, 437634035, No MRID - Vol. 6: Information Submitted Southern Regional Research Center for Non-target Plant testing. Request for waiver.

Gurian-Sherman, Doug and Tomimatsu, Gail. USEPA, OPP. June 23, 1999. BPPD Review, MRID 44464202: Review of Request for Waiver of Non-Target Organism Safety Effects Tests for EUP Renewal and Expansion for Biopesticide Containing *Aspergillus flavus* AF36 (AF36).

Gurian-Sherman, Doug and Tomimatsu, Gail. USEPA, OPP/BPPD. June 23, 1999a. BPPD Review, Additional Rationale for Data Waiver Request for Subdivision M Guidelines 154A-16, 17, 19, 20, 22, 23, 24. (No MRID).

Reynolds, Alan, Gail Tomimatsu and Zigfridas Vaituzis. USEPA, OPP. April 29, 2003. BPPD Review of MRID 45798102: Honeybee study.

Tomimatsu, G.S. 2003. USEPA, OPP. April 22, 2003. BPPD Review of Amendment Number 1 to MRID 45307202: *Aspergillus flavus* isolate AF36: Non-target Organism and Environmental Safety Information.

Tomimatsu, Gail and John Kough. USEPA, OPP/BPPD. May 15, 2003. BPPD Review of MRIDs 45307201, 45307202: Soil and Air Monitoring Studies and Product Performance Testing (Efficacy).

Tomimatsu, Gail S. and Vaituzis, Zigfridas. USEPA, OPP. May 15, 2003. BPPD Data Evaluation Record of MRID 45798102: Avian Inhalation Test, Tier I.