US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDE REGISTRATION ACTION DOCUMENT

Aspergillus flavus (NRRL 21882) (PC Code 006500)

March 24, 2004

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U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
***Aspergillus flavus* (NRRL 21882)**
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TABLE OF CONTENTS

Aspergillus flavus NRRL 21882 (PC Code 006500)

List of Tables

Tables are numbered according to the Section of the BRAD in which they appear.

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I. EXECUTIVE SUMMARY/FACT SHEET

Active Ingredient and Proposed Use

The active ingredient *Aspergillus flavus* NRRL 21882, also known as *A. flavus* NRRL 21882 or NPRL 45(PC Code 006500), belongs to the naturally occurring genus of fungi, *Aspergillus*, which is ubiquitous in the environment. This specific strain, *Aspergillus flavus* NRRL 21882 or NPRL 45, was isolated from a peanut seed in Georgia, United States (US) in 1991. It does not produce aflatoxins, cyclopiazonic acid or known intermediates in the aflatoxin biosynthetic pathway. When the pesticide is applied once per season to the soil at the pre-pegging phase of peanut plant growth, it is expected to displace aflatoxin-producing *A. flavus* strains naturally found on peanuts. Some other *Aspergillus* strains have been domesticated to provide human consumable products. For example, *Aspergillus oryzae* is used in the fermentation of soy sauce and miso, and the digestive enzyme alpha galactosidase found in Beano is produced from *Aspergillus niger*. NPRL 45 is not likely to exchange genetic material with other known aflatoxin producing strains of naturally occurring *A. flavus*, based on its characteristic lack of vegetative compatibility with the latter.

The Technical Grade Active Ingredient (TGAI), *Aspergillus flavus* NRRL 21882, EPA Reg. No. 75624-R, is manufactured in Japan (EPA Establishment # 75792-JPN-001). It is formulated in the US into the End-use Product (EP) afla-guard™ (EPA Reg. No. 75624-E), which contains 0.01% of the active ingredient, *Aspergillus flavus* NRRL 21882. The proposed application rate is 20 pounds EP (approximately 0.002 pound or less than 1 gram active ingredient) per acre. All manufacturing regulations must be met to assure the quality and integrity of the product. Quality control measures, discussed in **Section III. A.** of this BRAD**,** are in place to ascertain that human pathogens, potential metabolites, such as aflatoxins, and unintentional ingredients are within regulatory levels.

Toxicology, Human Exposure and Risks

Summaries of the toxicological effects, from reviews of submitted studies, are found in **Table III.B.2.c** (see **Section III.B.2.** of this BRAD**: Toxicology - Health Effects**). No toxic, infective or pathogenic effects were observed in two acute oral exposure tests in rodents. Based on submitted studies, both the TGAI and the End-use Product are considered Toxicity Category IV for acute oral effects. The results of the acute pulmonary exposure study showed no infectivity or pathogenicity, and clearance was observed from all tissues of surviving treated animals. Infective and pathogenic effects were observed in the intraperitoneal study and there was one unscheduled death, but the fungal active ingredient cleared all surviving rodent tissues by the end of the study on day 22. The implications of this study to pesticide handler exposure are summarized below under **Occupational and Residential exposure** and discussed in **Section III.B.4.** of this BRAD. No hypersensitivity incidents have been reported by workers who have conducted laboratory experiments and field trials for more than 11 years. The properties of *Aspergillus* species are known, and Personal Protective Equipment (PPE) are required to mitigate worker exposure.

Based on low toxicity potential observed in the studies, low application rates, no hypersensitivity reports and the clearance of the microbe from rodent tissues during the toxicology tests, data waiver requests for primary dermal, hypersensitivity and immune response studies were waived (see Section **III.B.2.** of this BRAD: Toxicology - Health Effects). The rationale for the request to waive data for the primary eye irritation study was supplemental, but upgradeable. However, the End-use Product is applied once during the season at approximately 1 gram of active ingredient per acre, and drift is expected to be minimal because of the adherence of the pesticide to the carrier. In addition, non-occupational eye exposure is not expected because of the application of the EP to commercial peanut fields and not residential areas. Provided eye protective equipment to mitigate eye exposure is on the label for the proposed use, this data waiver request is granted. Additional data or justification must be submitted to meet Agency guideline requirements, should the applicant wish to amend the registration to remove PPE for eye protection from the label.

Food Tolerances

This is the first proposed food/feed use of *Aspergillus flavus* NRRL 21882 for which an exemption from tolerance has been requested. The summaries of the reviewed studies, published literature and scientific and exposure rationales in support of this exemption from tolerance are included in this Biopesticide Registration Action Document (BRAD). A final rule establishing the exemption from tolerance for residues of *Aspergillus flavus* NRRL 21882 on peanut food and feed commodities will be published in the Federal Register concomitant with the issuance of the conditional registration of this pesticide.

FQPA Considerations

The Agency has considered *Aspergillus flavus* NRRL 21882 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 flavus* strains suggests that the fungus and its metabolites are normally expected to be present in/on food commodities regardless of treatment with *Aspergillus flavus* NRRL 21882 (**Section III.B.3** of this BRAD). Nevertheless, screening of starter cultures by Vegetative Compatibility assays, plating, and growth in an enrichment culture, ascertain product identity of the pesticidal microbial active ingredient.

The Agency also considered the potential for contamination by aflatoxins or unintentional ingredients associated with the pesticidal active ingredient, *A. flavus* NRRL 21882. Quality control and quality assurance methods are in place to ascertain that the pesticide itself is free of aflatoxins and that all batches containing aflatoxins and unintentional ingredients above regulatory levels must be incinerated or destroyed by appropriate technology. Lack of aflatoxins is determined by thin layer chromatography (TLC) or High Pressure Liquid Chromatography (HPLC). Kojic acid (KA), a metabolite associated with this group of fungi, is also found in other naturally occurring fungi, such as koji molds used for production of soy sauce. Levels of KA associated with this microbial active ingredient are much lower than the No Observed Adverse

Effects Level (NOAEL) of 250 mg/kg. There is no US Food and Drug Administration (US FDA) action level for KA, and the acute oral toxicology studies demonstrated low toxicity potential (Toxicity Category IV). Batches of the pesticide with potential contaminants or unintentional ingredients above regulatory levels are to be destroyed. Thus, contamination of peanuts by the pesticide itself, or by its metabolites, is not likely if quality control measures assure product integrity.

As discussed in detail in **Section III.B.2-9** of this BRAD, no toxicity endpoints were indicated to justify setting a numerical tolerance for *Aspergillus flavus* NRRL 21882. Based on the Toxicity Category IV classification for acute oral toxicity, and regulatory programs already in place that address *Aspergillus flavus* and aflatoxin residues on peanuts, a safety factor is not required for residues of *Aspergillus flavus* NRRL 21882 on peanuts. 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 as discussed in this document.

Dietary exposure via potential transfer of residues of *Aspergillus flavus* NRRL 21882, or its metabolites, to edible peanut food/feed commodities is not likely to pose an incremental risk above that which now exists from naturally occurring *Aspergilli* strains of fungi. Actually, treatment with the pesticide is likely to decrease aflatoxin levels in peanuts by 71-98% as demonstrated in efficacy trials **(see Section III.D.** of this BRAD**)**. Residues of *Aspergillus flavus* NRRL 21882 and its potential metabolites are not expected to survive the heating (blanching, roasting) associated with preparing edible peanuts or processing peanuts into its byproducts, peanut butter and peanut meal. The fungal active ingredient and potential metabolites are also not likely to separate into peanut oil due to the high heat and solvents used in processing. Such residues are also not expected to be different on peanut hay in treated fields than in untreated fields, because the total levels of *Aspergilli* in the soil do not change in the long-term following treatment with the pesticide **(Section III.D** of this document**).**

Furthermore, levels of *A. flavus* and potential metabolites of toxicological concern, such as the potent liver carcinogens, aflatoxins, on peanuts are regulated by the US Department of Agriculture (USDA) and the US FDA during marketing of peanut food/feed commodities **(Section III.B.3** of this BRAD**).** The pesticide is not intended for direct application to water or to crops grown in water, and runoff is expected to be minimal to non-existent based on application of the pesticide to drought-ridden areas or to peanut crops which are not generally irrigated. *Aspergillus flavus* NRRL 21882 is not registered on any other food/feed commodity, such that cumulative exposure is not expected . Thus, dietary exposure (including drinking water), cumulative and aggregate exposure of *Aspergillus flavus* NRRL 21882 to consumers via consumption of treated peanuts, peanut butter and peanut oil, and secondary transfer to meat and milk via peanut meal or peanut hay, are not expected to be greater, but may even be less, than current existing levels.

Occupational and Residential Exposure and Risk

While there was some infectivity and pathogenicity potential, and 1 unscheduled death, in the acute intraperitoneal (IP) study in rodents, clearance was observed from all tissues by the end of the 22 day study. Generally, FQPA considerations only take into account non-occupational exposure, but, in this case, the Agency considered the impact of potential effects via routes of exposure similar to the IP study in relation to both occupational (worker) and non-occupational exposure.

Potential exposure to workers and pesticide handlers of *A. flavus* NRRL 21882 is not expected to pose any incremental risk above that which currently exists. The pesticide demonstrates low toxicity, infectivity and pathogenicity potential by the acute oral or pulmonary routes. Occupational and non-occupational dermal and inhalation are expected, but mitigated as follows. Pesticide drift and non-occupational, as well as worker exposure, are minimized by (1) application as a large granular pesticide, in which the active ingredient adheres to the carrier; and (2) low application rates (less than 0.002 lb or approximately 1 gram of active ingredient per acre). Mitigation of exposure and risks to workers and pesticide handlers can be achieved by use of appropriate Personal Protective Equipment (PPE) and a Restricted-Entry Interval (REI) of 4 hours. In addition to the low exposure scenario noted above, non-occupational exposure is mitigated by application of the pesticide to commercial, agricultural sites, and not to residential areas. Thus, non-occupational or residential dermal and inhalation exposures are expected to be no greater than those expected from background *A. flavus* levels. **(Section III.B.4** of this BRAD**)**.

Ecological and Environmental Exposure and Risks

Ecological and environmental exposure and risk are summarized in **Section III.C.** of this BRAD. Evaluations of acceptable avian oral and inhalation infectivity/pathogenicity and honeybee exposure studies indicate low potential toxicity/pathogenicity effects of the pesticide and that the pesticide is not likely to pose any incremental adverse concerns to these non-target organisms. Data requirements were waived based on justifications that there were no recorded evidence of adverse effects to most non-target insects. In addition, total *A. flavus* levels do not increase following the single seasonal application of the pesticide. The low application rate of this naturally occurring soil colonizer to drought ridden peanut fields suggests minimal to no accumulation in water. Thus, the justifications to waive test data for freshwater fish, estuarine and marine vertebrates and invertebrates, and terrestrial non-target plants and endangered species are acceptable for the proposed uses.

Data Gaps and Requirements/Labeling

All deficiencies and labeling must meet Agency requirements **(Section V.C** of this document). Standard analysis of 5 production batches and efficacy data from a large scale field trial are required as conditions of registration **(Section VI** of this BRAD**)**. If more extensive use patterns are sought for treatment of other non-agricultural or agricultural sites or crops, additional information and data will be required on a case-by-case basis.

II. OVERVIEW

A. Product Overview

Biological Name: *Aspergillus flavus* NRRL 21882, *A. flavus* NRRL 21882, NPRL 45

ATCC/Culture Collection Number: National Regional Research Laboratories (NRRL) 21882 or National Peanut Research Laboratory (NPRL) 45

Trade and Other Names: *Aspergillus flavus* NRRL 21882 (TGAI or Manufacturing Use Product); afla-guard™ (End-Use Product)

OPP Chemical Code: 006500

Basic Manufacturer: Registrant:
Circle One
One Arthur Street; PO Box 28
Shellman, GA 39886-0028

Technical Grade Active Ingedient
Higuchi Matsunosuke Shoten
1-14-2, Harima-cho; Abeno-ku
Osaka; Japan 545-0022
EPA Establishment No. 75792-JPN-001

End-use Product
Circle One Global Inc.
1 Industrial Park Drive
Cuthbert, GA 39840
EPA Establishment No. 75624-GA-01

Consultant
Acta Group
1203 Nineteenth St. N.W, Suite 300
Washington D.C.20036-2401

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: Peanuts

Target Pests: Aflatoxin-producing fungi

Formulation Type: Granular.

Method and Rate of Application: Ground application at 20 pounds (0.002 lb or approximately 1 gram active ingredient) per acre. Sprinkle over the soil or apply in a band over crop rows by using a tractor-mounted Gandy box.

Use Practice Limitations: Apply prior to pegging of peanut plants, once per season.

Timing: The pesticide is applied once per year at the pre-pegging phase of peanut plant growth. Apply 40-80 days after planting, when abundant moisture is available, such as soon after a rain event. Ideally, the width of a peanut row, measured from the outer edges of foliage canopy, is about 18 inches. Peanuts are harvested about two to three months after treatment.

C. Estimated Usage

This is the first conditional registration of the active ingredient, so estimated usage data are not available.

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 *Aspergillus flavus* NRRL 21882, the product identity and analysis data, as well as the data and information submitted for acute mammalian toxicology and ecological effects, are sufficient to allow the proposed use patterns. Based on evaluations of submitted data, as discussed in this document, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of *Aspergillus flavus* NRRL 21882, as long as it is used as labeled.

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

(i) certifications of limits;
(ii) identification of *A. flavus* NRRL 21882 by taxonomy and VCG analysis or other appropriate method for enforcement purposes.
(iii) analysis and quantification of metabolites and other unintentional ingredients, including aflatoxins, cyclopiazonic acid (CPA) and kojic acid by appropriate enforcement analytical methods as required for quality assurance and quality control of the pesticide;
(iv) identification and enumeration of potential human pathogens and other microbial contaminants;
(v) storage stability; and
(vi) viability data.

All pesticides containing the active ingredient *Aspergillus flavus* NRRL 21882 that also contain unintentional ingredients, metabolites and contaminants above regulatory levels must be destroyed.

In addition, efficacy data are required from a large scale trial to include visual inspection of peanuts for fungal growth, and analysis of aflatoxin levels to ascertain the efficacy of the pesticide to meet USDA and FDA enforcement analytical standards. If more extensive use patterns are sought to include treatment of other 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

An application for an Experimental Use Permit and a pesticide petition, PP# 3G6559, for an exemption from temporary tolerance, was withdrawn on July 10, 2003. The registrant decided to complete studies to support other data requirements and submit a Section 3(c) registration application instead of doing field experiments. Data, which had already been collected from previous laboratory and small scale field trials, were submitted to the Agency in support of the Section 3(c) registration and evaluations are included in this BRAD.

Section 3(c) **Registration and Exemption from tolerance**

Section 3(c) Registration

EPA received an application from Circle One, One Arthur Street, P.O. Box 28, Shellman, GA 39886-0028 on January 20, 2004. The receipt of the application for the new active ingredient was published in the Federal Register, April 14, 2004 (Volume 69, Number 72)][Page 19845-19847](FRL- 7352-7]. No comments were received on this notice of receipt of application to register *Aspergillus flavus* NRRL 21882. This BRAD summarizes the data and information reviewed in support of the application and concludes that the pesticide is eligible for a conditional registration pending receipt of analyses of 5 production batches and efficacy data from a large scale efficacy field trial.

Exemption from Tolerance

Concomitant with the application for the Section 3(c) registration, the registrant filed a petition (PP # 4F6815) requesting a permanent exemption from the requirement of a tolerance for the active ingredient, *Aspergillus flavus* NRRL 21882, on peanuts. A notice of filing of this petition was published in the Federal Register, March 17, 2004 [Volume 69, Number 52; Page 12659-12664; FRL-7348-8].

EPA received 7 comments in response to the Notice of Filing. Six of those comments were from farmers who support the use of *Aspergillus flavus* NRRL 21882 to reduce aflatoxin contamination of peanuts. Among their comments in support of the pesticide, these farmers noted the tremendous cost, in excess of $25 million dollars per year, to manage aflatoxin contamination of peanuts. The Agency is working expeditiously to evaluate the data submitted to support registration of the active ingredient *Aspergillus flavus* NRRL 21882. The Final Rule granting an exemption from the requirement of a tolerance, and this BRAD are part of that process.

The seventh comment raised a number of issues and concerns. First, the commenter objected to the publication of the applicant's data summaries submitted with the petition prior to EPA's evaluation of such data and viewed the Notice of Filing as an attempt to obtain approval with insufficient information. This commenter appears to misunderstand the nature and purpose of a Notice of Filing. Under Section 408(d)(3) of the FFDCA, EPA is required to publish a notice of the filing of a petition seeking the establishment of a tolerance or an exemption from the requirement of a tolerance. That notice must contain an applicant-prepared "informative summary" of the data, information, and arguments provided by the applicant in support of its petition. (See FFDCA § 408(d)(2)A)(i)(I)). The Notice of Filing is published in the Federal Register prior to the Agency's evaluation of the petition and the data submitted in support of that petition. Once EPA has evaluated the petition and all supporting data, EPA issues a final rule, such as this one, which includes EPA's assessment of the applicant's submissions, as they relate to dietary risk, and EPA's determination vis-a-vis the requested tolerance or tolerance exemption. The Notice of Filing, in and of itself, is not an indication of whether the sought tolerance or tolerance exemption will, in fact, be granted by the Agency.

Second, the commenter objected to the applicant's animal test reports and the number and duration of the studies underlying those reports, and to the applicants' requests to waive data. With respect to the animal tests, the commenter also suggested that human cell testing or testing on humans should be done instead. EPA regulates pesticides according to peer-reviewed and publicly available guidelines that describe endpoints for human health risk assessment. Tests are conducted with the active ingredient or end-use product in surrogate animals, through various routes of administration (i.e., oral, dermal, pulmonary, etc.). Any effects seen are reported to the Agency, peer-reviewed, and evaluated to determine whether the effects of the test material demonstrate infectivity, acute toxicity, or pathogenicity. While tests in some human cell-lines are available, they may not always be applicable, and may not assist the Agency in making as accurate an assessment of the hazards and risks posed by the use of the pesticide as can be done with surrogate animal tests. Both positive and adverse effects are reported by the applicant so that toxicological concerns for human health and environmental risk assessment can be identified and mitigated according to sound scientific practice and taking into account the exposure levels and risks associated with the pesticide. If further testing is required to fully evaluate any hazard and risks posed by the test material under proposed use patterns, the registrant must submit the appropriate additional data to satisfy EPA's published guideline requirements. EPA does not deviate from these guidelines without good reason, and does so for data waiver requests only when sound scientific consensus on the provided data waiver rationale is reached. In this case, and as discussed more thoroughly below **(Sections III.B. and III.C.** of this BRAD**)**, EPA granted the requested waivers only after determining that the rationales provided in support of those waiver requests were acceptable.

Third, the commenter asserted that dermal sensitivity to this product is already known to exist, and that more of it is not needed. While there is a potential for dermal sensitivity to the *Aspergillus* group of fungi, the specific pesticide at issue here, *Aspergillus flavus* NRRL 21882, is not intended for residential applications. Instead, it is to be applied to commercial agricultural

fields in accordance with the requirements of the applicable Worker Protection Standards. Workers are protected from potential dermal and inhalation exposure to the pesticide by appropriate Personal Protective Equipment (PPE) as required on the label **(see Section VI** of this document**)**. Pesticide drift is not expected from the application of the granular End-use Product which is applied at a very low rate (approximately 1 gram or 0.002 pound of active ingredient per acre). Thus, non-occupational residential exposure is expected to be minimal to non-existent, and occupational exposure is mitigated **(see Section III.B.4** of this BRAD**).**

Finally, the commenter objected to the statement by the applicant that this application is not likely to increase the natural concentration of *Aspergillus* in water, and thus is not considered to be a risk for drinking water. As discussed below, EPA's evaluation of the acute oral studies conducted in rodents indicates no toxicity or pathogenicity via oral exposure to this pesticide, which includes exposure via drinking water **(see Section III.B.5** of this BRAD**)**. Furthermore, this pesticide is not applied directly to water, but to the soil in drought ridden regions where accumulation in water is not likely to occur. In addition, *Aspergillus flavus* NRRL 21882 is expected to displace native aflatoxin-producing *Aspergillus* fungi at the sites of application, thus reducing the potential hazards posed by these ubiquitous toxigenic fungi. For a more complete discussion of EPA's findings regarding *Aspergillus flavus* NRRL 21882 and drinking water, (see **Section III.B.5.** of this document).

EPA has thus addressed the comments received in response to the Notice of Filing and the summary of the petition contained therein seeking an exemption from the requirement of a tolerance for *Aspergillus flavus* NRRL 21882. The remainder of this document and the Final Rule to be published simultaneously with this decision, summarize the Agency's review and consideration of the tolerance exemption and registration requests. The low toxicity potential as demonstrated in the acute oral studies and potential dietary exposure and risk are discussed below **(Section III.B.** of this BRAD**).** The submitted data and information support the exemption from tolerance for residues of *Aspergillus flavus* NRRL 21882 and its end-use product, afla-guard™ on peanuts.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

The data submitted in support of product identity requirements for *Aspergillus flavus* NRRL 21882 are sufficient for the proposed use patterns of the microbial pesticide.

1. Product Identity and Mode of Action

Product Identity

Technical Grade Active Ingredient (TGAI)

Aspergillus flavus NRRL 21882, also called NPRL 45, is a non-aflatoxin-producing strain of *Aspergillus flavus* fungi which are ubiquitous in the environment. This specific strain was isolated from a peanut seed at the USDA National Peanut Research Laboratory in 1991. *Aspergillus flavus* NRRL 21882 belongs to the vegetative compatibility group (VCG) 24, which is a sub-population of *Aspergillus flavus* from which no isolates, so far, have been shown to produce aflatoxins or cyclopiazonic acid. This naturally occurring strain acts as a microbial pest control agent by displacing other aflatoxin-producing strains of *A. flavus* from the target crop. Corresponding residues of *A. flavus* NRRL 21882 are identified by lack of ability to produce aflatoxins and cyclopiazonic acid, and by Vegetative Compatibility Group (VCG) typing. After 7 days of incubation on a solid agar matrix, identification of *Aspergillus flavus* NRRL 21882 conidia is performed by microscopic observation (MRID 45884001, BPPD Data Evaluation Review (DER) dated July 16, 2003a, hereafter referred to as BPPD DER 07/16/2003a).

Colony color, texture, and other morphological characteristics identify the fungal active ingredient, and the same plates are examined microscopically after 4 days to check for fungal contaminants and obtain a viable count of the TGAI (MRID 45884001, BPPD DER 07/16/2003a). A discussion of product characterization, quality assurance and quality control (QA/QC) measures to assure the quality of the pesticide product, and to ascertain that unintentional ingredients, metabolites, and potential contaminants are within regulatory levels, is presented below.

Taxonomy

Based on morphological characteristics, *A. flavus* NRRL 21882 is assigned to the *Aspergillus flavus* genus and species, but belongs to the strain NRRL 21882. On samples taken from the environment *A. flavus* NRRL 21882 can be detected by the yellow-green young spores borne in chains on a stipitate vesicle. When isolated on media at 37 °C, colonies appear after 2 to 3 days. Identifying *Aspergillus flavus* NRRL 21882 conidia is performed after 7 days on Czapek agar at 30 °C by observation of colony color, texture, and other morphological characteristics. Colonies remain green and do not shift to brown on Czapek's agar, where conidia are echinulate (otherwise they are smooth to slightly roughed), and can have single but mostly double sterigmata with radiate and very loose columnar heads. Conidia are $\leq$ 6.4 um diameter, conidiophores are < 800 um long on average, and colonies are deeply velutinous to lightly floccose (MRID 46196801; BPPD DER 05/06/2004a").

Unintentional Ingredients and Potential Metabolites

Apart from taxonomic characterization, *Aspergillus flavus* NRRL 21882 is mainly characterized by its lack of aflatoxins as determined by laboratory tests. As mentioned earlier, *Aspergillus flavus* NRRL 21882 belongs to vegetative compatibility group (VCG) 24. So far, no isolates from this sub-population of *Aspergillus flavus* have been shown to produce aflatoxins or cyclopiazonic acid. Analyses of aflatoxin B_2, and cyclopiazonic acid are routinely conducted by the TGAI manufacturer. Testing for these and other aflatoxins occurs again prior to end-use product formulation. These analytical methods are acceptable to assay batches of *Aspergillus flavus* NRRL 21882 conidia for aflatoxins, cyclopiazonic acid, bacterial contaminants, and bacterial pathogens (MRID 46196801; BPPD DER 05/06/2004a). These methods can be used to monitor the pesticide to ascertain that product identity and integrity meet Agency requirements.

Chloroform extracts of the TGAI incubated in an enrichment broth for 7 days are analyzed for metabolites. Lack of aflatoxins B_1, B_2, G_1, G_2 and cyclopiazonic acid in the TGAI is shown by thin-layer chromatography. Analyses of the TGAI for aflatoxin B_1 and pathogenic bacteria were performed by Japan Food Research Laboratories. No aflatoxin B_1 (5 ppb limit) was detected. No aflatoxins or cyclopiazonic acid were detected (MRID 45884001, BPPD DER 7/16/2003a). To confirm their absence, additional HPLC assays to detect aflatrem, dihydroxyflavinine, paspalinine, sterigmatocystin, aspergillic acid, 3-nitropropionic acid and versicolorin A were performed on separate cultures. These substances were not detected (MRID 46196801; BPPD DER 05/06/2004a).

A. flavus NRRL 21882 was shown to produce kojic acid (KA), which is also produced by *Aspergillus oryzae* (koji molds) during fermentation of food products for human consumption. There is no FDA action level for kojic acid in foods. The published oral No Observed Adverse Effect Level (NOAEL) is 250 mg/kg from subchronic animal exposure studies (Gerhard J. Nohynek, 2004), while some other reports for the LD_{50} values for KA may vary depending on the route of exposure, and the types of study and test animals. Endogenous levels of KA in the pesticide are reported by the applicant to be in the microgram range far below the published oral NOAEL values. Though residues of kojic acid on untreated, or *Aspergillus flavus* NRRL 21882 treated, peanuts have not been established,(MRID 46196801; BPPD DER 05/06/2004a), it may already be present in peanuts due to presence of natural populations of *Aspergillus* and other kojic acid producing fungi in soil.

Monitoring by enrichment cultures demonstrated no *Escherichia coli* (in 2.22 g), no *Salmonella* (in 25 g) and no *Vibrio parahaemolyticus* (in 0.1 g) in conidia of *A. flavus* NPRL45, which is an alternate name given to this active ingredient (MRID 45884001, BPPD DER dated 7/16/2003a). Two batches of conidia tested for the absence of the pathogenic bacteria showed that bacterial contaminants, *E. coli*, *Salmonella*, *Vibrio parahaemolyticus*, are within regulatory levels(MRID 46196801; BPPD DER 05/06/2004a). All pesticides containing *Aspergillus flavus* NRRL 21882 with unintentional ingredients and potential metabolites above regulatory levels must be destroyed.

End-Use Product

Hulled barley is coated with conidia previously subjected to quality assurance tests to meet regulatory standards for unintentional impurities, potential metabolites, human pathogens and microbial contaminants (MRID 45884001, BPPD DER dated 7/16/2003a). A study to characterize the EP (MRID 45884001) reviewed in 2003 was considered **SUPPLEMENTAL but upgradable** pending submission of additional information regarding the manufacturing process, certified limits, unintentional metabolites, and storage stability (MRID 45884001, BPPD DER dated 7/16/2003a). Another submission in 2004 (MRID 46196801; BPPD DER 05/06/2004a) provided most of the information required but was also considered **supplemental but upgradable,** pending resolution of the Material Safety Data Sheets (MSDS), certified limits, and storage stability (BPPD DER 05/06/2004a).

Further information submitted by the applicant provided MSDS and clarified certified limits. The inerts are acceptable and exempt from the requirement of a tolerance according to 40 CFR 180.950(a) and 40 CFR 180.1001 (BPPD Memorandum dated May 28, 2004a). **Tables III.A.1a and 1b** summarize the product characterization studies evaluated in support of this conditional registration decision. As a condition of registration analysis of 5 production batches must be submitted to satisfy guideline requirements (**Section VI** of this BRAD). Manufacture of the EP must meet the requirements of Occupational Safety and Health Administration (OSHA) and all other relevant manufacturing regulations.

Table III.A.1.a: Product Characterization - *A. flavus* NRRL 21882 (TGAI) and afla-guard™ (EP).

Guideline	Study	Result	MRID #
151-10 *885.1100	Product Identity	Taxonomy (morphology), Vegetative Compatibility Group and metabolite analysis for identification of *A. flavus* NRRL 21882. Acceptable**.	45884001 46196801
151-11 *885.1200	Manufacturing Process	Acceptable**	45884001 46196801
151-12 *885.1300	Discussion of Formation of Unintentional Ingredients	Acceptable**. Absence of aflatoxins,CPA and bacterial human pathogens demonstrated in 3 batches. Quantification of unintentional ingredients, metabolites (including aflatoxins and kojic acid), bacterial and fungal contaminants in 5 production batches and required.	45884001 46196801
151-13 *885.1400	Analysis of Samples	Acceptable for two batches**; 5 production batch analysis required as condition of registration.	45884001 46196801
151-15 *885.1500	Certification of limits	Acceptable**. Units by weight, and information on all ingredients must be included.	46196801 BPPD memo 5/28/2004a.
151-16	Analytical Method	Taxonomy (morphology) and Vegetative Compatibility Group analysis for identification of *A. flavus* NRRL 21882; lack of aflatoxins and CPA. Acceptable**.	46196801

*OPPTS Harmonized Guidelines
Acceptable for decision that pesticide is eligible for conditional registration. Analysis of 5 production batches required as condition of registration **(see Section VI of this BRAD**)**.

Mode of Action

The life cycle of *Aspergillus flavus* NRRL 21882 upon release and in culture is documented in several submitted publications. *A. flavus* NRRL 21882 is intended to grow after release to the field during periods of increased moisture after peanut plants are actively growing. The intent is that NRRL 21882 will displace toxigenic *A. flavus* strains and colonize the peanut during pegging or below ground (possibly by vector transmission) if conditions favorable to natural *Aspergillus flavus* infection are present during the growing season - namely drought conditions without sufficient irrigation, or presence of nematode or insect vectors that penetrate the peanut shell. The registrant has submitted preliminary, unpublished data showing NRRL 21882 remains viable on peanut seed after harvest (MRID 46196801; BPPD DER 05/06/2004a).

2. Physical And Chemical Properties Assessment

Table III.A.1.b: Physical & Chemical Properties of *A. flavus* NRRL 21882 (TGAI) and afla-guard™ (EP).

Guideline	Property	*Aspergillus flavus* NRRL 21882 (MP)	MRID #	afla-guard™ EP	MRID #
63-2 *830.6302	Color	green	46196801	brown (color of whole grain hulled barley)	46196801
63-3 *830.6303	Physical state	Solid powder	45884001 46196801	Solid	46196801
63-4 *830.6304	Odor	Odorless	45884001 46196801	Odor like barley	45884001 46196801
63-17 *830.6317	Storage stability	> 3 years ---------- > 6 years in sealed nylon-polyethylene bags at 5°C	45884001 ---------- 46196801	Periodic retesting of stored spores ranged from 1.0 - 2.7 x 10^{10} CFU/g.	46196801
63-19 *830.6319	Miscibility**	Not applicable	46196801	Not applicable	45884001 46196801
63-20 *830.6320	Corrosion characteristics**	Not corrosive	46196801	Not corrosive	45884001 46196801
63-12 *830.7000	pH**	Not applicable	46196801	Not applicable	45884001 46196801
63-2 *830.7100	Viscosity**	Not applicable	46196801	Not applicable	45884001 46196801
63-7 *830.7300	Density or specific gravity	0.52 ± 0.02 g/cm	46196801	0.83 ± 0.02 g/cm - by weighing spores	45884001 46196801

* OPPTS Harmonized Test Guidelines

**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, explodability, viscosity, miscibility, and dielectric breakdown voltage were not required because of the known properties of the solid fungal ingredient and granular nature of the microbial pesticide.

3. Analytical Methods for Peanuts and Pesticide

Why analytical method is not required for peanut residue data

Residue data for *Aspergillus flavus* NRRL 21882 or its metabolites on peanuts as a result of treatment by the pesticide are not required for the exemption from tolerance which is included in the eligibility decision in this BRAD. *Aspergillus flavus* NRRL 21882 occurs naturally in the soil and may be associated with peanuts regardless of pesticide treatment. Thus, there is a great likelihood of prior exposure for most, if not all, individuals and the increase in exposure due to

this proposed microbial pesticide would be negligible. In addition, it likely is not possible to differentiate between the naturally occurring residues of *Aspergillus flavus* NRRL 21882 and those residues attributable to *Aspergillus flavus* NRRL 21882, the pesticide. Moreover, the acute oral studies discussed below demonstrate that the active ingredient does not pose a dietary risk. For these reasons, the Agency has concluded that an analytical method to detect residues of this pesticide on peanuts for enforcement purposes is not needed.

However, treated peanut food/feed commodities, must meet the requirements for aflatoxins and metabolites as regulated by the Food and Drug Administration and the United States Department of Agriculture. In this respect, and because aflatoxin is considered a public health hazard requiring efficacy data, analysis of peanuts is required to demonstrate that the pesticide is efficacious.

Analysis of pesticide samples

The Agency has concluded that for analysis of the pesticide itself, the methods discussed above **(Section III.A.** of this BRAD**)** are acceptable for enforcement purposes for product identity of *Aspergillus flavus* NRRL 21882 (VCG analysis) and its metabolites (TLC and HPLC). VCG analysis and nutrient utilization tests are used to screen starter cultures to identify the non-aflatoxin-producing *Aspergillus flavus* NRRL 21882 strain. Starter cultures of *Aspergillus flavus* NRRL 21882 are also selected on the basis of the lack of aflatoxin as monitored by standard thin layer chromatography (TLC) and HPLC procedures. Other appropriate methods are required for quality control 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. Summaries of the data evaluated in support of the product characterization of the TGAI and EP are summarized below in **Tables III.A.1a and III.A.1b.**

Condition of Registration - Product Characterization

The data summarized above are acceptable for the conditional registration of the Technical Grade Active Ingredient, *Aspergillus flavus* NRRL 21882, and its End-use Product afla-guard™ pending submission of the standard five production batch analysis.

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

(i) certification of limits;
(ii) identification of *A. flavus* NRRL 21882 by taxonomy and VCG analysis;
(iii) analysis and quantification of metabolites and other unintentional ingredients such as aflatoxins, CPA and kojic acid in the pesticidal active ingredient;
(iv) identification and enumeration of microbial contaminants and potential human pathogens;
(v) storage stability; and
(vi) viability data.

B. Human Health Assessment

1. Food Clearances/Tolerances

An exemption from tolerance for *Aspergillus flavus* NRRL 21882 is being established with this eligibility for a conditional registration of the pesticide for use on peanuts. Residues of NRRL 21882 or its metabolites are not expected to be different from natural background on the food/feed commodity, peanuts. Summaries of eight field trials, were reported to the Agency to support the claim that *Aspergillus flavus* NRRL 21882 reduces aflatoxins in field-grown peanuts by 71 to 98%. These studies were conducted either with *A. flavus* NRRL 21882, as proposed here, or in conjunction with another *A. flavus* strain **(Section III.D** of this BRAD; BPPD DER, 05/05/2004a**)**. Aflatoxins are potential metabolites of the aflatoxin-producing strains but not of *A. flavus* NRRL 21882. Regardless of treatment with *A. flavus* NRRL 21882, peanut food/feed commodities for human and animal consumption are subject to compliance with the regulatory levels of fungus and aflatoxin contamination as routinely monitored by the US Department of Agriculture (USDA) and the US Food and Drug Administration (US FDA).

Thus, there is a reasonable certainty that no undue adverse effects are likely to result from exposure to products treated with *A. flavus* NRRL 21882. This includes all anticipated dietary exposures and all other exposures for which there is reliable information, as long as the pesticide is used according to label directions. Below is the toxicology assessment **(Section III. B.2.** of this BRAD**)**, and discussion of other factors, which led to this conclusion **(Section III. B.3 - 9.** of this BRAD**)**.

2. Toxicology Assessment

Summaries of acute toxicological and pathogenicity studies **(Table III.B.2.c)** and the rationales for certain data waiver requests (**Table III.B.2.c)** are discussed below.

a. Acute Oral Toxicity [MRID 45884002; OPPTS 870.1100, Guideline 81-1; and MRID 46196802; OPPTS 885.3050, Guideline 152-30]

Two studies were submitted in support of these guideline requirement**s**. In an acute oral toxicity study (MRID 45884002), young adult rats (5 per sex) were dosed by gavage with a single dose of 5000 mg per kg of a test substance in corn oil after they had fasted overnight. The test substance was composed of barley coated with 50 % *A. flavus* NRRL 21882 and 50% of another *Aspergillus* species. Body weights were measured one day prior to dosing (Day 0), just prior to dosing (Day 1), and on Days 7 and 14. Animals were observed for mortality and clinical signs approximately 1, 2.5 and 4 hours after dosing, then once daily for 14 days following treatment. There were no mortalities, or gross abnormalities on necropsy. Anogenital staining, soft feces, and/or colored material around the nose was observed in some animals to Day 2. The male and female LD_{50} is > 5000 mg per kg. This study was considered acceptable for the test material used, (Biopesticide and Pollution Prevention Division (BPPD) Data Evaluation Review (DER) 05/06/2004c) and for the End-use Product because the inerts are the same as those proposed for the EP, afla-guard™.

Another acute oral toxicity/pathogenicity exposure study was conducted on the TGAI where the test material was *A. flavus* NRRL 21882 only (MRID 46196802). This second study, included 23 male and 23 female young adult rats. The rats were divided into the following groups:

Groups 1-4 = Treated groups, all received treatment of 2.35-3.80 x 10^8 CFU/rat.
Group 5 = Controls treated with test material which was autoclaved 121°C for 15 minutes.
Group 6 = Untreated shelf controls; held in same room as treated groups.
Group 7 = Untreated non-shelf controls; held in a separate room.
Group 8 = Sterile culture filtrate control; held in same room as non-shelf controls.

The test material was administered by a single oral gavage to treated groups, which were then observed over a 22 day period, and with sacrifices at specific intervals. Sacrificed rats were subjected to necropsy in the order of non-shelf then shelf controls followed by the treated groups. Recovery of viable *Aspergillus flavus* NRRL 21882 from blood, organs, intestinal contents, and feces was determined by serial decimal dilution with peptone saline tween (PST) and incubation at 30-35 °C for a minimum of 48 hours.

All animals gained weight during the study and there were no unscheduled deaths. No treatment-related clinical signs were observed, and no abnormal findings were noted at any necropsy interval. *Aspergillus flavus* NRRL 21882 was not detected in any organ or blood sample. The rate of clearance of viable *Aspergillus flavus* NRRL 21882 was not determined since insufficient viable test organisms were recovered from test samples. Based on the presented/submitted data, the test organism was not toxic, infective, or pathogenic to rats by oral administration and was classified as **ACCEPTABLE**. The acute oral LD_{50} was greater than 2.35-3.80 x 10^8 CFU/rat and the pesticide was classified as Toxicity Category IV on the basis of these studies (BPPD DER, 05/06/2004b).

b. Acute Pulmonary Toxicity/Pathogenicity [MRID 45884003; OPPTS 885.3150]

In a 22-day acute pulmonary toxicity pathogenicity study, young adult rats (17 per sex) were administered a suspension of *Aspergillus flavus* NRRL 21882 in a single dose by intratracheal instillation at 5.77 - 7.20 x 10^7 CFU per animal. No mortalities or evidence of pathogenicity due to *A. flavus* NRRL 21882 were seen. Transient respiratory signs (rales and/or irregular respiration) were observed in some treated rats up to 1 hour post-dosing. A single mortality on Day 2 probably was not due to *A. flavus* NRRL 21882 and may have been caused by the mechanism of dosing. There was no evidence of treatment related effects on body weight or temperature, or that *A. flavus* NRRL 21882 proliferated or was infective in treated rats. As expected from the test procedure, viable *A. flavus* was recovered in lung tissue in 5 of 6 animals sacrificed 1 hour post-dosing (10^2 - 10^6 CFU per g tissue) and in the lungs of the single rat that died on Day 2 (10^4 CFU per g). No viable organisms were found in any other tissues or organs examined during the remainder of the study. *A. flavus* was reported in feces of 2 of 5 males studied (12 and 357 CFU per g) and 3 of 5 females studied (10, 77, and 64,400 CFU per g) only on Day 4 and this was thought to occur from active muco-ciliary lung clearance of *A. flavus*

NRRL 21882. The LD_{50} for pulmonary exposure was considered greater than 5.77 - 7.20 x 10^7 CFU per animal. The rate of clearance of viable *A. flavus* NRRL 21882 from tissues was not calculated because no viable organisms were recovered in any sample past the day of dosing, except from lungs of a single mortality on day 2. Aspergillosis is commonly associated with *Aspergillus fumigatus* in the majority (approximately 90%) of cases and, on rare occasions with some strains of *A. flavus*. However, this study, showing pulmonary clearance indicates that Aspergillus flavus NRRL 21882 is not likely to be a causative agent in aspergillosis. This study was considered **ACCEPTABLE** (MRID 45884003, BPPD DER 07/16/2003a).

c. Acute Inhalation [MRID 45884003; OPPTS Guideline 152-32]

Based on the known properties of *Aspergillus flavus*, Agency-required respiratory protection for pesticide applicators, and the low toxicity potential from pulmonary exposure in the test described above (MRID 45884003; OPPTS 885.3150, BPPD DER, 07/16/2003), an acute inhalation study was not required pursuant to 40 CFR §158.740(c)(i). The acute pulmonary toxicity study immediately above demonstrated a low toxicity potential for *A. flavus* NRRL 21882 in the lungs. The LD_{50} for pulmonary exposure was considered greater than 5.77 - 7.20 x 10^7 CFU per animal (**Table III.B.2.c**, **Section III.B.2** of this BRAD**)**. To minimize inhalation exposure, the TGAI is to be slurried under laminar flow conditions and loaded into closed, automated systems for formulation into the granular EP, consisting mainly of hulled barley (approximately 93%). While *A. flavus* NRRL 21882 conidia may be less than 10 micron in size, hulled barley particles to which the conidia adhere, are larger than 10 microns and are not themselves respirable.

Furthermore, the low rates of application and the single, seasonal ground application, minimize occupational and non-occupational inhalation exposure, as discussed below in **Section III.B.4** of this BRAD**.** Based on the acute pulmonary study, the nature of the inerts, and Agency-required respiratory protection for pesticide applicators, an acute inhalation study on *A. flavus* NRRL 21882 is not required for this proposed use. A dust/mist filtering respirator with NIOSH prefix N–95, R-95 or P-95 is required to mitigate against occupational exposure because of the microbial nature of the pesticide. Non-occupational inhalation exposure to commercial, not residential areas, is discussed in **Section III.B.4** of this BRAD**.**

d. Intravenous, Intracerebral, Intraperitoneal injection [MRIDs 45884004 and 46223901; OPPTS 885.3200, Guideline 152-33]

In an acute injection toxicity/pathogenicity study, young adult rats (3 per sex) were injected IP with a single dose-suspension of *Aspergillus flavus* NRRL 21882 in Tween 80 at approximately 10^7 CFU per animal. All active substance-treated animals died or were sacrificed for humane reasons on Day 5 - 6 when treated animals showed severe clinical signs (i.e. piloerection, hunched posture, abnormal gait or reduced body tone and underactive behavior) with lack of a pyrogenic response. Animals treated with heat-inactivated or live *A. flavus* NRRL 21882 (i.e. white nodules and adhesions on a number of organs). High levels (> 10,000 CFU per g) of *A. flavus* NRRL 21882 were found in the spleen or liver of animals that died naturally and

from the sole animal sacrificed on day 5. $LD_{50} < 10^7$ CFU per animal. The study was considered **SUPPLEMENTAL**. The claimed lack of infectivity in moribund or deceased rats is inconclusive due to an unknown etiology so the test should be repeated with appropriate test animals, treatments and controls (BPPD DER 07/16/2003). The registrant consulted with EPA scientists on the protocol prior to repeating this test.

A second study, submitted in January 2004 (MRID 46223901), was conducted with 22 male and 22 female rats. Treated groups received 1.13 - 1.47 x 10^7 CFU/rat *Aspergillus flavus* NRRL 21882 without Tween 80, by intraperitoneal injection. One of the control groups received a sterile culture filtrate and other controls received either autoclaved test material, or no treatment. Animals were observed over a 22 day period. The results of this study, showing recovery of viable Aspergillus flavus NRRL 21882, are presented in **Tables III.B.2.a and III.B.2.b.**

No test organisms were detected in any samples from the controls. Viable NRRL 21882 was below detection (<10 CFU/mL) in blood at all sample times. At one hour after dosing, the test organism was detected in the kidneys, spleen, liver, heart, lungs, mesenteric lymph nodes and intestinal contents of treated rats, but was below detection (<10 CFU/mL) in the brain. By day 4, viable counts were still high in the spleen but decreased in other organs, while low levels of viable NRRL 21882 are found in the brain of 3 out of 6 rats. By day 8, clearance was observed from all tissues in the males, and from most tissues except the spleen and mesenteric lymph nodes of females, which cleared by day 22. Clearance from intestinal contents and feces occurred in males prior to day 8, and in females by day 22. After the 22 day period, clearance had occurred from all tissues and samples (MRID 46223901).

One female (No. 32) in Group 4 treated with viable NRRL 21882 was sacrificed on day 7 because of severe clinical effects. No unscheduled deaths were observed in any other group. Lower overall mean body weight gains in 1 group were not considered due to the viable test organism, but may have been attributable to experimental fecal sampling procedures only performed on this group (BPPD Review dated May 6, 2004a).

Table III.B.2.a: Recovery of *Aspergillus flavus* NRRL 21882 in samples from male rats treated IP.					
	Sacrifice Day and Mean Viable (CFU/g) Recovery				
	1[a]	**4**	**8**	**15**	**22**
Brain	<10	<10 - 30	<10	<10	<10
Kidneys	17,400 - 233,000	31 - 299	<10	<10	<10
Spleen	29,500 - 158,000	1,800 - 50,600	<10	<10	<10
Liver	4,680 - 6,350	49 - 72	<10	<10	<10
Heart	17 - 26	<10 - 12	<10	<10	<10
Lungs	186 - 2,500	<10 - 42	<10	<10	<10
Mesenteric lymph nodes	10,000 - 19,900	578 - 1,180	<10	<10	<10
Intestinal contents	170 - 9,820	<10	<10	<10	<10
Feces	<10 - 12	n/a	<10	<10	<10

Table III.B.2.b: Recovery of *Aspergillus flavus* NRRL 21882 in samples from female rats treated IP.					
	Sacrifice Day and Mean Viable (CFU/g) Recovery				
	1[a]	**4**	**8**	**15**	**22**
Brain	<10	<10 - 28	<10	<10	<10
Kidneys	10,500 - 34,300	45 - 57	<10	<10	<10
Spleen	45,900 - 163,000	1,020 - 18,000	<10 - 688	14 - 255	<10
Liver	18,000 - 95,000	102 - 205	<10	<10	<10
Heart	11 - 212	<10	<10	<10	<10
Lungs	248 - 4,240	30 - 211	<10	<10	<10
Mesenteric lymph nodes	23,500 - 74,000	116 - 469	<10 - 11	<10 - 18	<10
Intestinal contents	161 - 7,770	<10 - 325	<10	<10 - 11	<10
Feces	<10 - 19	n/a	<10 - 18	<10	<10

Clinical signs noted starting on days 7 to 22 post-dosing in 1 of 6 males and 3 of 6 females (including a female rat euthanized at day 7) are head tilting or leaning [3 of 12 treated], abnormal gait [1 of 12 treated], repetitive head turns and/or circling [2 of 12 treated], and limited use of rear limbs [1 of 12 treated]. The study director concluded head tilting and circling in 1 male, and head tilting in 1 female were probably related to the viable test organism (BPPD Review dated May 6, 2004a). Clinical signs did not clear from 3 of 6 remaining animals at study termination on day 22. Based on this study, *Aspergillus flavus* NRRL 21882 was considered infective and pathogenic to rats by intraperitoneal administration; IP $LD_{50} > 1.13 - 1.51 \times 10^7$ CFU/rat.

While the results of this IP test suggest potential infectivity via serious injury as reflected by an intraperitoneal route of exposure, it is important to note that clearance was observed from all tissues of surviving animals in this IP study, a finding consistent with all the other toxicology

studies reported above. More importantly, the results of this IP test, while relevant to issues of occupational exposure, are not as relevant to the tolerance exemption determination, which focuses on non-occupational exposure. Indeed, the acute oral studies reported above, which are directly relevant to an analysis of dietary, non-occupational exposure, indicate no infectivity or pathogenicity. In addition, if the pesticide is used as labeled (approximately one gram active ingredient per acre), much lower levels of non-occupational exposure are expected when peanuts are consumed than can be extrapolated from the IP test, in which the test substance was administered directly into the abdominal cavity at a rate of 10^7 CFU/animal.

Moreover, the pesticide is not to be applied to residential areas, but rather only to commercial peanut fields, and any potential pesticide residues on treated peanuts are further mitigated by processing as described in **Section III.B.3** of this BRAD**.** Furthermore, the inerts are food grade and cause the active ingredient to adhere to the carrier (hulled barley), thus minimizing pesticide drift or transfer of residues. Finally, and as mentioned previously, *Aspergillus flavus* strains occur naturally in the environment and non-occupational or residential exposures are expected to be no greater than that expected from background *Aspergillus flavus* levels. All of these factors and considerations minimize non-occupational exposure and allow the Agency to conclude that the dietary risks posed by the use of this pesticide are likely to be minimal and that there is a reasonable certainty that no harm will result from use of this microbial agent **(Section III.B.4** of this BRAD).

It should be clarified, however, that in connection with the Agency's consideration of *Aspergillus flavus* NRRL 21882 for purposes of registration, as distinct from the toxicology data submitted for a tolerance exemption action, the Agency has considered the worst case scenario in which similar types of IP occupational exposures may occur. The relevance of this IP test is to seriously injured workers or to those who may come in contact with the pesticide through a similar route of exposure intraperitoneally. As previously stated, the granular pesticide is applied at a very low rate to the soil with little or no pesticide drift. Worker exposure is minimized by the use of Personal Protective Equipment (PPE) that includes long sleeve shirt, long pants, shoes, socks, waterproof gloves, eye protection and an appropriate dust/mist filtering respirator with the NIOSH prefix N –95, P-95, or R-95. Early-entry workers, engaged in post-application activities, must wear this PPE when entering treated fields during the 4 hour Restricted-Entry Interval (REI) **(Section III.B.4** of this BRAD**)**.

Summaries of the previously discussed toxicology studies evaluated are presented in **Table III.B.2.c**. below.

Table III.B.2.c: Summary Tier I Acute Mammalian Toxicity - Aspergillus flavus NRRL 21882 and afla-guard™

Guideline	Study	Toxicity Category	Results	MRID #
81-1 *870.1100	Acute oral toxicity	IV	LD_{50} >5000 mg/kg. Test substance contained 50% *A. flavus* NRRL 21882 and same inerts as afla-guard™. No mortality/clinical signs observed over the 14 day study. **Acceptable for EP**	45884002
152-30 *885.3050	Acute oral toxicity/ pathogenicity	N/A	LD_{50} > 2.35 - 3.80 x 10^8 CFU/rat. No mortality or treatment-related clinical signs were observed, and no abnormal findings were noted at any necropsy interval. Viable NRRL 21882 cleared from the gastrointestinal tract prior to 15 days. **Acceptable for TGAI or MP.**	46196802
Satisfies **152-32** *885.3150 (see below)	Acute pulmonary toxicity/ pathogenicity	N/A	LD_{50} > 5.77- 7.20 x 10^7 CFU per animal. No treatment related mortality or evidence of pathogenicity in rats in the 22 day study. Viable NRRL 21882 cleared from lungs prior to day 4 and from feces prior to day 8. **Acceptable, TGAI, EP.**	45884003
152-32 *870.1300	Acute inhalation	N/A	Not required based on pulmonary study, non-respiratory nature of inerts, and respiratory protection required for pesticide applicators. Minimal inhalation exposure from slurry and closed systems during manufacture.	45884003
152-36 *885.3400	Hypersensitivity Incidents	N/A	No hypersensitivity incidents reported by lab or field trial workers. Agency requires reports of adverse effects and hypersensitivity incidents to comply with 6(a)(2) 40CFR159.152. **Acceptable, TGAI, EP.**	46196804 BPPD DER 05/06/2004c
152-33 *885.3200	Intraperitoneal injection toxicity/ pathogenicity	N/A	Similar post-mortem findings were observed in animals treated with live and heat-inactivated *A. flavus* using Tween 80. Substance-treated animals died or were sacrificed for humane reasons on Day 5 - 6. Lack of infectivity in moribund or deceased rats is inconclusive due to an unknown etiology. **Supplemental but upgradable, TGAI, EP.**	45884004

Table III.B.2.c: Summary Tier I Acute Mammalian Toxicity - Aspergillus flavus NRRL 21882 and afla-guard™				
Guideline	**Study**	**Toxicity Category**	**Results**	**MRID #**
152-33 *885.3200	Intraperitoneal injection toxicity/ pathogenicity	N/A	Of 22 male and 22 female rats, one unscheduled death on day 7 occurred from viable NRRL 21882 without Tween 80. IP administration led to rapid dissemination to all organs, including the brain, but no detection in blood. Clearance of viable NRRL 21882 by day 22 from organs, though symptoms did not clear from all surviving animals. Infective and pathogenic; IP $LD_{50} > 1.13 - 1.51 \times 10^7$ CFU/rat. **Acceptable, TGAI, EP.**	46223901

*** OPPTS Harmonized Guideline Numbers.**

e. Hypersensitivity Incidents [MRID 45739104; Guideline 152-37]

Personnel at the USDA Agricultural Research Service National Peanut Research Laboratory have been working with different strains of *Aspergillus flavus* since 1987 and have performed numerous studies in laboratory and field settings with the active ingredient, *A. flavus* NRRL 21882, with no reported adverse effects. There are no data that suggest this strain is more or less likely to induce hypersensitivity than other naturally occurring *A. flavus* strains (MRID 46196804; BPPD DER 05/06/2004c). However, in the future and in order to comply with FIFRA Section 6(a)(2) requirements under 40CFR159.152 and OPPTS 885.3400, any incident of hypersensitivity associated with the use of this pesticide must be reported to the Agency.

f. Data Waivers

A. Acute Oral for EP [MRID 45884003 OPPTS *870.1100; Guideline 81-1*]

A request was submitted to waive data for the acute oral toxicity/pathogenicity for the End-use Product, afla-guard™, based on the acceptable results of the acute oral toxicity/pathogenicity studies conducted with the TGAI (summarized above) and the nature of the inerts, which are exempt from the requirement of a tolerance according to 40 CFR 180.950 (a); 40 CFR 180.1001. Since the EP contains 0.01% of the TGAI, this rationale was acceptable to the Agency and the data requirement for the acute oral toxicity/pathogenicity study for the EP was waived (BPPD DER, May 28, 2004). Furthermore, an acceptable study in support of acute oral toxicity/pathogenicity (MRID 45884003; see **Section III.B.2.a** of this BRAD) was conducted with an EP which contained the same inerts as proposed for afla-guard™ and 50% *Aspergillus flavus* NRRL 21882. No other data are required for this guideline for acute oral toxicity/pathogenicity for this EP. If the formulation changes, additional data may be required on a case-by-case basis.

B. Data waivers were also requested for the following studies for both the TGAI or MP and the EP.

i. Acute Dermal Toxicity/pathogenicity [OPPTS Harmonized Guideline 885.3100; Guideline 152-31]
ii. Primary Dermal Irritation [OPPTS Harmonized Guideline 870.2500; Guideline 152-34]
iii. Primary Eye Irritation [OPPTS Harmonized Guideline 870.2400; Guideline 152-35]
iv. Hypersensitivity Study [OPPTS Harmonized Guideline 870.3400; Guideline 152-37]
v. Immune Response [OPPTS Harmonized Guideline 880.3800 Guideline 152-38]

Application of the EP, hulled barley inoculated with *Aspergillus flavus* NRRL 21882, for the guideline tests to study primary dermal irritation for the EP is impractical. Furthermore, non-occupational dermal or inhalation exposure, or exposures via any of the routes covered by the guideline studies listed directly above, are expected to be no greater than that which occurs naturally for the following reasons. In mixing/loading and application experiments, spores of the pesticide are not released from the carrier and did not increase in the air space (MRID 46196804; BPPD DER dated May 06, 2004c, hereafter referred to as BPPD DER 06/06/2004c). In addition, data from an unpublished study showed that the total level of *Aspergillus* strains in the soil increases after product application, but then declines and stabilizes, and that the total amount of *Aspergillus* strains in the crop is unaffected (MRID 46196804; BPPD DER 06/06/2004c). Thus, levels of *Aspergillus* strains are not expected to be greater than those which normally and naturally exist as a result of treatment of peanut fields with this pesticide.

Data from the toxicology tests reported above indicate no toxicity or pathogenicity when the active ingredient is administered orally or via the pulmonary route. And while there is the potential for infectivity or pathogenicity after intraperitoneal injection, that study also demonstrates clearance of the test organism from all tissue samples by the end of the study. Results from these supporting toxicology tests indicate that test mammalian immune systems can clear the organism (see **Section III.B.2.a-d** of this BRAD). In addition, no adverse effects were reported by workers or researchers who handled the active ingredient during the experimental phase. Moreover, the pesticide is applied at a low rate of approximately 0.9gram to 1 gram active ingredient per acre once during the growing season, and the use of PPE will protect workers from exposure to the pesticide (see **Section III.B.4** of this BRAD). Based on these considerations, the justifications in support of the request to waive data for acute dermal toxicity/pathogenicity (OPPTS 885.3100), primary dermal irritation (OPPTS 870.2500), the hypersensitivity study (OPPTS 870.3400), and immune response (OPPTS 880.3800) were acceptable (BPPD DER 05/06/2004c)**.**

The rationale for the request to waive data for the primary eye irritation study was supplemental but upgradable (BPPD DER 05/06/2004c). The End-use Product is applied once during the season at approximately 1 gram of active ingredient per acre, and drift is expected to

be minimal because of the adherence of the pesticide to the carrier. Provided eye protective equipment to mitigate eye exposure is on the label for the proposed use, this data waiver request is granted. Additional data or justification must be submitted to meet Agency guideline requirements, should the applicant wish to amend the registration to remove PPE for eye protection from the label. Summaries of the status of the data waiver requests are summarized in **Table III.B.2.d** below.

Table III.B.2.d: Tier I - Data Waivers: Acute Mammalian Toxicity of *Aspergillus flavus* NRRL 21882 (TGAI) and afla-guard™ (EP)

Guideline	Study	Comments	MRID No.
81-1 *870-1100	Acute Oral toxicity/pathogenicity	Results of TGAI acute oral toxicity studies support EP. Also acceptable study with test material conducted with 50% A. flavus NRRL 21882 and same inerts as afla-guard™. **Waived, EP**	45884003
152-31 *885.3100 **152-34** *870.2500 **152-36** *870.2600	Acute dermal toxicity Primary dermal irritation Dermal sensitization	Not toxic/infective by oral and pulmonary routes. Belongs to fungal group with known dermal sensitizers. Long sleeve shirt, long pants, shoes, socks, waterproof gloves, goggles, respirator and 4 hour REI required for workers. Non-occupational dermal exposure mitigated by commercial, agricultural uses only, minimal pesticide drift and low exposure. **WAIVED**, TGAI, EP.**	45884002 45884003 46196802
152-35 *870.2400	Primary eye irritation	**SUPPLEMENTAL** - TGAI slurried to minimize exposure prior to automated manufacturing. For EP low eye exposure based on adherence of pesticide to granules, and requirement for eye protective PPE. If registrant wishes to amend label to remove eye PPE, acceptable waiver rationale or study is required. **WAIVED**, TGAI, EP.**	
152-37 *870.3400	Hypersensitivity Study	**WAIVED**, TGAI, EP.** Acceptable report of no hypersensitivity incidents (gdln 152-36). **Agency requires reports of adverse effects and hypersensitivity incidents to comply with 6(a)(2) 40CFR159.152.**	BPPD DER 05/06/2004c
152-38 *880.3800	Immune Response	Clearance in tox/path studies indicate organism clears via mammalian immune systems. **WAIVED**, TGAI, EP**	45884002 45884003 46196802

***OPPTS Harmonized Guideline Numbers. ** Waived for TGAI and EP, if pesticide is used as labeled.**

g. 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.

h. 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* NRRL 21882, 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 acute oral, pulmonary and intraperitoneal toxicity/pathogenicity studies, 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 use of this 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

As discussed above, *A. flavus* NRRL 21882 is neither toxic nor infective as determined by oral exposure studies in rats, when dosed by oral gavage at 2.35-3.80 x 10^8 CFU/animal (MRID 46196802; BPPD DER 05/06/2004a). A safety net already exists in that treated commodities for human and animal consumption must meet fungal and aflatoxin contamination regulatory levels set by the US Department of Agriculture (USDA) and the US Food and Drug Administration (US FDA). In addition, summaries of eight field trials were reported to the Agency to support the

claim that *Aspergillus flavus* NRRL 21882 reduces aflatoxins in field-grown peanuts. Aflatoxins were measured in shelled and unshelled peanuts by High Pressure Liquid Chromatography (HPLC). Five of the trials used the active ingredient in combination with another *A. flavus* strain and did not use the product label application rate. The remaining three multi-year efficacy studies of small plot field trials demonstrate that aflatoxin is reduced by 71 to 98% in peanuts treated with *A. flavus* NRRL 21882 (MRID 46196805, BPPD DER dated May 5, 2004, hereafter referred to as BPPD DER 05/05/04a). In addition, in the acute oral study discussed above, the reported LD_{50} is greater than 5000 mg/kg rat body weight. No mortality, toxicity or infectivity was associated with the TGAI in this study **(Section III.B.2.** of this BRAD**)**.

Residues of the active ingredient, *A. flavus* NRRL 21882, are not likely to survive the processing associated with making edible peanut products. Information submitted on oil extraction reveals high heat and solvents are used that would kill viable fungi and also probably remove any toxins during processing. All known uses of peanuts for food use require roasting, either after shelling, or whole. EPA expects that any food uses for peanuts will result in processing steps that also will kill any viable *Aspergillus flavus* that may end up in food, whether naturally or through use of afla-guard™ (BPPD DER, May 06, 2004ac). Residues of the active ingredient and its potential metabolites on peanut hay are not expected to be different in treated fields than in untreated fields.

These data support the claim that dietary exposure to treated peanuts will likely reduce exposure to aflatoxins, which are potent liver toxins and carcinogens. Kojic acid (KA) is likely already present in peanuts due to presence of natural populations of *Aspergillus* in soil and is present in products fermented by koji molds for human consumption. Though residues of kojic acid on untreated, or *Aspergillus flavus* NRRL 21882 treated peanuts have not been established, the published oral No Observed Adverse Effect Level of 250 mg/kg (Gerhard J. Nohynek, 2004) observed in subchronic animal studies suggests low toxicity potential. Both the oral toxicity/pathogenicity and intraperitoneal toxicity/pathogenicity exposure studies used a seven day broth culture cell free extract treatment, in addition to live and heat-killed NRRL 21882 treatments, and demonstrated no acutely toxic or pathogenic effects to rats to this extract. Thus, the Agency is of the opinion that dietary exposure from the metabolite kojic acid is not likely to pose any undue dietary hazards. Dietary exposure via drinking water, as presented below (**see 5**), does not pose any incremental hazard. Therefore, the Agency has decided that dietary exposure to *Aspergillus flavus* NRRL 21882 is not likely to result in any undue health effects.

4. Occupational and Residential Exposure and Risk Characterization

a. Residential, School, Daycare and Medical Facility Exposure and Risk

Non-occupational dermal and inhalation exposure is not likely to be greater than that which normally exists from naturally occurring *Aspergillus flavus* strains, as discussed below. This determination is based on several rationales. The pulmonary study demonstrated that the pesticidal active ingredient is not infective to mammals when instilled into rats intratracheally (see **Section III. B.2**. of this BRAD). Non-occupational or residential dermal and inhalation exposures are expected to be no greater than background *A. flavus* levels. As discussed above,

pesticide drift is expected to be minimal based on the granular nature of the pesticide, and a formulation in which the active ingredient is expected to adhere to the carrier. Finally, lack of reports of hypersensitivity incidents during the experimental phase, and return of levels of *Aspergillus flavus* to background shortly after germination (see Section III.C. of this BRAD), suggest that non-occupational dermal and inhalation hazards will be minimal.

b. Occupational Exposure and Risk

Similarly, low application rates to soil either by tractor-mounted Gandy box or similar equipment will not pose undue occupational exposure and risk to workers and pesticide handlers if the pesticide is used as labeled. Appropriate PPE and a 4 hour REI will mitigate occupational exposure and risk. PPE for mixer/loader, applicator and other pesticide handlers, and restricted-entry post-application workers, include long sleeve shirt, long pants, shoes, socks, waterproof gloves, goggles and a dust/mist filtering respirator with the NIOSH prefix N–95, R-95 or P-95.

5. Drinking Water Exposure and Risk Characterization

Exposure to *Aspergillus flavus* NRRL 21882 in drinking water is not likely to be greater than current/existing exposures to *A. flavus* strains. Potential risks via exposure to drinking water or runoff are adequately mitigated by, among other things, percolation through soil. The pesticide is to be applied to drought ridden areas to decrease and displace proliferation of natural aflatoxin-producing strains. The pesticide is not for application to crops grown in water, and, if used as labeled, is not likely to accumulate in drinking water. Thus, exposure via drinking water from proposed use of this non-aflatoxin-producing strain of *A. flavus* is not likely to pose any incremental risk to adult humans, infants or children. In fact, displacement of toxigenic strains of *A. flavus* by this non-aflatoxin-producing strain may decrease exposure to aflatoxins, which are potent liver toxins and carcinogens. Thus, exposure from the proposed use of *A. flavus* NRRL 21882 is not likely to pose any incremental risk via drinking water to adult humans, infants or children.

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

Results from Tier I studies did not trigger Tier II subchronic or Tier III chronic dietary exposure studies. Based on submitted studies, the TGAI, *Aspergillus flavus* NRRL 21882, demonstrates low acute oral toxicity potential, and was classified as toxicity category IV for acute oral effects (BPPD DER May 06, 2004b). This microbial pesticide is intended for use on peanuts. It was isolated from a peanut seed in Georgia and is expected to be found there after treatment. *Aspergillus flavus* NRRL 21882 is not expected to survive the heating and solvents associated with processing peanuts into edible commodities, or other measures used to mitigate aflatoxin contamination. Moreover, starter cultures of the TGAI are screened for lack of aflatoxins, according to studies submitted to the Agency **(Section III.A** of this BRAD). Aflatoxins in peanuts and its byproducts, e.g. peanut butter, peanut oil and peanut meal, must meet the regulatory levels for fungus and aflatoxin contamination as required by the USDA and US FDA. These considerations led the Agency to conclude that the acute, subchronic and chronic risks

posed by dietary exposure to the pesticide via use on peanuts are not likely to be any greater than those which currently exist from exposure to natural *Aspergillus flavus* strains.

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

Dermal

Potential non-occupational dermal exposure to *Aspergillus flavus* NRRL 21882 is unlikely because the use sites are commercial, agricultural and because of the granular nature of the pesticide, which minimizes pesticide drift. As discussed earlier **(see Section III.B.2 & 4** of this BRAD**)**, lack of hypersensitivity incidents, low application rates and return of *Aspergillus flavus* levels to background shortly after germination, poses minimal risk to populations via non-occupational dermal exposure, which is expected to be no greater than the existing background exposure to natural *A. flavus* strains (see **Section III.B.4.a** of this BRAD**)**.

Oral

Sections **III B.2.** (Toxicology), **III.B.3.** (Dietary exposure and Risk) **III.B.5.** (Drinking Water) and **III.B.6.** of this BRAD discuss the rationales behind the Agency's determination that consumption of peanuts and its byproducts treated with *A. flavus* NRRL 21882 is not likely to pose any incremental risk over that which currently exists.

Inhalation

As discussed in **Section III.B.4.** of this BRAD, non-occupational inhalation exposure is expected to pose no undue hazard to human adults, infants and children when the pesticide is used as labeled. This determination was made on the basis of the low application rate of the granular pesticide to the soil once during the growing season, and minimal expected pesticide drift.

In summary, the potential aggregate exposure via treatment of peanuts with *A. flavus* NRRL 21882 is not likely to pose any incremental hazard above that which currently exists from background *A. flavus* strains already present in the agricultural environment. This includes hazards derived from (a) dietary exposure from the treated food/feed commodity, peanuts; (b) from drinking water potentially exposed secondary to treatment of sites with this pesticide; and (c) dermal and inhalation exposure of populations to *A. flavus* NRRL 21882.

8. Cumulative Effects

Section 408(b)(2)(D)(v) of the FFDCA requires the Agency to consider the cumulative effect of exposure to *Aspergillus flavus* NRRL 21882 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. Based on tests in mammalian systems, *Aspergillus flavus* NRRL 21882 does not appear to be toxic or pathogenic to humans. Another non-aflatoxin-producing strain, *Aspergillus flavus* AF36, is conditionally registered for use on cotton, but not on peanuts. There are no other registered pesticide products containing *Aspergillus flavus* NRRL 21882, and other *Aspergillus flavus* strains abound naturally in the environment. Moreover, the displacement of the aflatoxin-producing strain of *Aspergillus flavus* by *Aspergillus flavus* NRRL 21882 may reduce aflatoxin contamination of peanuts. Based on the low toxicity potential of *Aspergillus*

flavus NRRL 21882, the fact that it is non-aflatoxigenic, and the safety net already in place to monitor food/feed commodities for aflatoxins (see **Section III.B.2** of this BRAD), no cumulative or incremental effect is expected from the use of *Aspergillus flavus* NRRL 21882 on peanuts.

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

There is reasonable certainty that no harm will result to the U. S. population, including infants and children, from aggregate exposures to residues of *Aspergillus flavus* NRRL 21882, as a result of its use as an antifungal agent on peanuts. 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 harm, from this fungus in its use as an antifungal agent on peanuts via dietary exposure since the organism is non-toxic and non-pathogenic to animals and humans. 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 above,** in this BRAD**)**. Moreover, non-occupational inhalation or dermal exposure is expected to be no greater than that which currently exists **(see Section III.B.4** of this BRAD**).**

FFDCA Section 408(b)(2)(C) provides that EPA shall apply an additional ten-fold margin of exposure (safety) for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data base on toxicity and exposure, unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety), which are often referred to as uncertainty factors, are incorporated into EPA risk assessment either directly, or through the use of a margin of exposure analysis, or by using uncertainty (safety) factors in calculating a dose level that poses no appreciable risk. In this instance, based on all the available information (as discussed in detail above), the Agency concludes that the fungus, *Aspergillus flavus* NRRL 21882, is non-toxic to mammals, including infants and children. Because there are no threshold effects of concern to infants, children and adults when *Aspergillus flavus* NRRL 21882 is used as labeled, the Agency has determined that the additional margin of safety is not necessary to protect infants and children, and that not adding any additional margin of safety will be safe for infants and children. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of *Aspergillus flavus* NRRL 21882.

C. Environmental Assessment

1. Ecological Effects Hazard and Risk Assessment

The studies and data waiver justifications submitted for *Aspergillus flavus* NRRL 21882 are sufficient to allow its conditional registration as a microbial pesticide for use on peanuts. Below is a summary of the ecological effects data and data waiver requests evaluated in support of this conditional registration action **(see also Table III.C.1.a and III.C.1.b)**.

a. Toxicity to Terrestrial Animals

i. Avian Oral Toxicity/Pathogenicity [MRID 46240701; OPPTS 885.4050; Guideline 154-16]

The registrant submitted a request for a data waiver for the Avian Oral Acute Toxicity Study (USEPA Microbial Testing Guidelines OPPTS 885.4050). However, the Agency requested that the study be performed. A study was conducted and submitted to the EPA for review (MRID 46240701). In the submitted study, toxicity and pathogenicity of *A. flavus* NRRL 21882, to young Bobwhite quail (*Colinus virginianus*) was determined by oral intubation at a mean daily dose of 3.97 $x10^8$ CFU/kg of body weight per day for five days, followed by a 30 day observation period. The dosage corresponded to a total of approximately 1.89 $\times 10^9$ CFU/kg of body weight. No treatment-related mortalities or signs of illness were observed with the exception of one accidental death in the infectivity/vehicle control. No evidence of toxicity or pathogenicity was observed. The no mortality dosage of *A. flavus* NRRL 21882 administered to Bobwhite quail in the study was approximately 3.97 $x10^8$ CFU/kg of body weight per day for five days (BPPD DER dated May 05, 2004a hereafter referred to as "BPPD DER 05/05/2004a."). No further study is required for this guideline for this proposed use of *A. flavus* NRRL 21882 on peanuts.

ii. Avian Intratracheal Injection [MRID 45884005, OPPTS 885.4100; Guideline 154 -17]

The study in Bobwhite quail, which was submitted in support of the avian intratracheal injection study, was acceptable for this guideline (MRID No. 45884005; BPPD DER dated May 06, 2004d, hereafter referred to as BPPD DER 05/06/2004d).

No deaths resulted from the test material administered to young Bobwhite quail for 5 consecutive days and observed over 30 days. The LD_{50} is greater than 8.64 x 10^5 CFU per bird per day, or greater than 2.82 x 10^8 CFU per Kg per day. No clinical signs nor evidence of toxicity, pathogenicity or infectivity were evident during the 5-day dosing or 30-day observation period. Vehicle control birds exhibited no apparent response to treatment and remained healthy during a preliminary test. There were no mortalities or signs of toxicity, pathogenicity or infectivity detected in any birds. No effect on body weight or food consumption was observed. No grossly observable lesions were found at necropsy. These results indicate that *A. flavus* NRRL 21882 is not toxic, pathogenic or infective to Bobwhite quail when tested at a maximum hazard dose (MRID No. 45884005; BPPD DER dated July 2003 hereafter referred to as BPPD DER 07/16/2003b). Aspergillosis is commonly associated with *Aspergillus fumigatus* in the majority (approximately 90%) of cases and, on rare occasions, with some strains of *A. flavus*. However, the submitted avian studies, showing clearance from all analyzed tissues, indicate that *Aspergillus flavus* NRRL 21882 is not likely to be a causative agent in aspergillosis of avian non-target organisms.

In summary, the submitted acceptable avian oral acute toxicity and avian intratracheal injection studies (MRID Nos. 46240701 and 45884005 respectively) are sufficient to demonstrate that exposure and risk are not expected to avian wildlife from proposed uses of *A. flavus* NRRL 21882. No further studies are required for these guidelines for these proposed uses of the TGAI and EP.

iii. Wild Mammal Testing: Acute Toxicity/Pathogenicity [MRIDs 45884002, 46196802, 45884003; OPPTS 885.4150; Guideline 154A-18]

These data are required only when the acute oral rodent toxicity/pathogenicity study (OPPTS 885.3050) is not sufficient for wild mammal hazard assessment. The acute oral rat study submitted in support of the registration is sufficient to make a no apparent hazard finding to wild mammals(MRID No. 46196802). The study authors concluded that viable *A. flavus* NRRL 21882 demonstrated no toxicity or pathogenicity when administered in a single oral dose of 2.35-3.80 x 10^8 CFU/rat. According to this study, the acute oral LD_{50} is greater than 5000 mg/kg. Similarly, the acute pulmonary study in rodents demonstrated no infectivity or pathogenicity (LD_{50} greater than 5.77 x 10^7 CFU per animal. While the intraperitoneal study demonstrated some infectivity and pathogenicity, clearance was observed in all analyzed tissues of surviving rodents at the end of the 22 day study. Only 1 unscheduled death occurred in that study.

Aspergillosis is commonly associated with *Aspergillus fumigatus* in the majority of cases and, on rare occasions, with some strains of *A. flavus*. However, the submitted studies, showing clearance from all analyzed tissues in surviving mammals, indicate that *Aspergillus flavus* NRRL 21882 is not likely to be a causative agent in aspergillosis of mammals. Based on the low observed mammalian toxicity/pathogenicity effects in the acute oral and pulmonary toxicity/pathogenicity tests in rodents (MRIDs 45884002, 46196802, and 45884003; **Section III. B.2.** of this BRAD) the Agency has decided that use of this microbial pesticide is not likely to pose incremental hazards to wild mammals if used as labeled. No additional testing at higher tiers is required.

iv. Beneficial Insects; Honeybee Testing [MRID 45884006; OPPTS 885.4380; Guideline 154-24]

The registrant submitted a whole-hive honey bee field exposure study with *Aspergillus flavus* NRRL 21882 (MRID No. 45884006) to fulfill the Microbial Non-target Organisms Data Requirements outlined in 40 CFR 158.740 (USEPA OPPTS 885.4380). The honey bees were exposed to a 20 lb/acre application of afla-guard™-treated alfalfa (as a surrogate crop) over a 30-day period in a single field, while an adjacent untreated field served as a control. There was no significant difference in mortality observed between the test and control groups in any measure, using Analysis of Variance (ANOVA) statistical analyses. The pesticide, afla-guard™, was rated non-hazardous to honey bees. The study was reviewed and determined to be **supplemental but upgradable** to acceptable with the submission of information addressing irrigation practices/field moisture during the study and information pertinent to observations for the bee larval disease 'stonebrood', reportedly caused by some *Aspergillus flavus* strains (MRID No. 45884006; BPPD DER July 16, 2003b, hereafter referred to as BPPD DER 07/16/2003b).

The registrant submitted a supplement (MRID No. 46196806) to the initial study that has adequately demonstrated that afla-guard™ germinated during the study. The study authors irrigated the field after treatment application and did plate counts from soil samples taken from the field before and after treatment. The registrant cited that stonebrood is a larval disease that is

visually apparent and the study director confirmed that if stonebrood was observed, they would have noted it in the study report. Therefore, EPA's questions regarding the honey bee test for *A. flavus* NRRL 21882 (MRID No. 45884006; BPPD DER May 05, 2004c, hereafter referred to as BPPD DER 05/05/2004c) have been sufficiently answered and the study is upgraded to **acceptable** (BPPD Review May 06, 2004d).

Table III.C.1.a: Eco-Toxicology Summary/Studies Evaluated

Guideline No.	Study	Status, Classification & Comments	MRID Nos.
154-16 *885.4050	Avian Oral Toxicity	The no mortality *A. flavus* NRRL 21882 oral dose to Bobwhite quail is > 3.97 x10^8 CFU/kg body weight per day for five days with a 30-day observation period. No treatment-related signs of illness were observed. **Acceptable.**	46240701
154-17 *885.4100	Avian inhalation	The no mortality *A. flavus* NRRL 21882 intratracheal inhalation dose to Bobwhite quail is > 2.82 x 10^8 CFU/Kg body weight per day for five days with a 30-day observation period. No treatment-related signs of illness were observed. **Acceptable.**	45884005
154-18 *885.4150	Wild Mammal Testing	No hazards from *A. flavus* NRRL 21882 for wild mammalian species are anticipated for this use. The acute oral and pulmonary rat studies are sufficient to make a no apparent hazard to wild mammals finding. **Acceptable.**	45884002 46196802 45884003
154-24 *885.4380	Honey Bee Testing	No hazards from *A. flavus* NRRL 21882 for honey bees are anticipated for this use. There was no significant difference in mortality observed between *A. flavus* NRRL 21882 treated and control groups exposed in similar fields. afla-guard™ was rated non-hazardous to honey bees. **Acceptable.**	45884006 46196806

*OPPTS Microbial Pesticide Harmonized Test Guideline Numbers.

b. Toxicity to Aquatic Animals, Non-target Insects, and Non-target Plants Data Waivers: Ecological Effects

The registrant provided justifications to support a request to waive the following ecological effects studies:

i. **Freshwater Fish Testing** [OPPTS 885.4200; Guideline 154-19]
ii. **Freshwater Aquatic Invertebrate Testing** [OPPTS 885.4240; Guideline 154-20]
iii. **Estuarine and Marine Animal testing** [OPPTS 885.4280; Guideline 154-21]
iv. **Non-target Plant Studies** [OPPTS 885.4300; Guideline 154-22]
v. **Non-target Insect Testing** [OPPTS 885.4340; Guideline 154-23]

Justifications for Data Waivers

The justifications advanced the following arguments: 1) the active ingredient is a naturally occurring soil colonizer, 2) a published literature search found no relevant recorded evidence of adverse effects on non-target organisms, 3) the product is applied to soil once a year and submitted data citations indicate that the increased concentrations in the soil are temporary, 4) the potential for reaching bodies of water is very low because peanut fields are not generally irrigated and the formulation is granular, 5) phytotoxicity in non-target plants has not been observed in the years of testing the product for use on various crops. The waiver requests were reviewed by the Agency and the results of the assessment are presented here in both tabular (**Table III.C.1.b.**) and more detailed descriptive format.

The following rationales, summarized below, justify data waiver requests for these guideline tests for non-target organisms. The literature citations provided in the data waiver justifications show that *A. flavus* NRRL 21882 occurs naturally in the environment among the various strains of *A. flavus*. Other submitted data demonstrate that naturally occurring populations of total *A. flavus* have been reported to vary from 0.5 to $>10^5$ CFU/g and may vary significantly (greater than 10-fold) within a single field in a year. A three-year field study conducted by the USDA National Peanut Research Laboratory (NPRL) demonstrated that the total *A. flavus* concentration increased with the application of *A. flavus* NRRL 21882 but declined by the spring of the following year. Similarly, a submitted unpublished study found that there was an increase in the total population of *A. flavus* after application of afla-guard™ but after years of testing, the total amount of *A. flavus* in the soil did not increase in the long term. Since the concentration of natural populations of *A. flavus* are shown to widely fluctuate, the increased concentration of *A. flavus* NRRL 21882 post application should not produce effects other than those seen from natural *A. flavus* population fluctuations (BPPD Review 05/06/2004d).

The EP, afla-guard™ is a granular formulation which minimizes the potential for drift. It would be applied to the soil of peanut fields once a season. Thus, runoff from fields adjacent to bodies of water would be expected to be low. The proposed use suggests that the level of *A. flavus* NRRL 21882 in the aquatic environment will not significantly increase (BPPD Review 05/06/2004d).

Additional justifications are summarized below for relevant guidelines:

i. Freshwater Fish Testing [OPPTS 885.4200; Guideline 154-19]

ii. Freshwater Aquatic Invertebrate Testing [OPPTS 885.4240; Guideline 154-20]

The registrant provided a search of published literature to demonstrate that there are no reports of adverse effects to freshwater fish or aquatic invertebrates due to natural populations of *A. flavus* NRRL 21882 (BPPD Review 05/06/2004d). The data provided indicates that the proposed uses of afla-guard™ should not pose a hazard for freshwater fish and aquatic invertebrates (BPPD Review 05/06/2004d). The justifications are **acceptable** to waive these data requirements.

iii. Estuarine and Marine Animal Testing [OPPTS 885.4280; Guideline 154-21]

Data for this guideline are conditionally required only when the product is intended for direct application to the estuarine and marine environment, or is expected to enter this environment in significant concentrations because of the intended use or mobility pattern. The EP, afla-guard™ is intended for use in peanuts, and is a granular formulation which minimizes the potential for drift. Therefore, the risk of runoff into an estuarine or marine environment should be minimal. Furthermore, the registrant provided a search of published literature to demonstrate that there are no reports of adverse effects to estuarine and marine animals due to natural populations of *A. flavus* NRRL 21882 (BPPD Review 05/06/2004d). The data and information provided are **acceptable** and indicate that the proposed uses of afla-guard™ should not pose a hazard for estuarine and marine animals.

iv. Non-target Plants - Terrestrial and Aquatic [OPPTS 885.4300; Guideline 154-22]

The registrant states that many efficacy studies have been conducted in greenhouse and field trials for *A. flavus* NRRL 21882 over more than a decade with no phytotoxic effects observed. *Aspergillus flavus* NRRL 21882 naturally occurs in the soil among various other strains of *A. flavus*. Because this product is expected to be used mainly in drought-ridden regions, the risk of increase in *A. flavus* NRRL 21882 exposure to aquatic non-target plants via runoff is not likely. Terrestrial plants would also have limited to no exposure to afla-guard™ because it is a granular product that is ground applied and would not be subject to spray drift. A literature search conducted by the registrant did not reveal citations related to plant toxicity for the strain being considered for registration. There were references found for some strains of *A. flavus* being highly pathogenic to cotton seedlings and as causing albinism in sweet orange and grapefruit seeds (when inoculated with strains of *A. flavus* known to cause albinism in maize). However, these phytotoxic effects were reported from undefined *A. flavus* strains in general and not for *A. flavus* NRRL 21882 specifically. The NRRL 21882 strain does not produce aflatoxins, cyclopiazonic acid, or known intermediates in the aflatoxin biosynthetic pathway. The **acceptable** data and information provided indicate that the proposed uses of afla-guard™ should not pose any incremental hazard to non-target plants that does not already exist from naturally occurring *A. flavus* strains (BPPD Review 05/06/2004d).

v. Non-target Insect Testing [OPPTS 885.4340; Guideline. 154-23]

The granular formulation, afla-guard™, minimizes the potential for movement via water or air. It would be applied to the soil of commercial peanut fields once a season and runoff from fields adjacent to bodies of water would be expected to be low. A published literature search submitted yielded no reports of adverse effects to non-target insects due to *A. flavus* NRRL 21882. The literature reveals that some members of the *A. flavus* group have been implicated in bee paralysis (stonebrood), in diseases of silkworms, of *Diadasia bituberculata*, of locusts, of the clover leaf weevil, and of subterranean termites. However, these references were for undefined *A. flavus* strains and not for *A. flavus* NRRL 21882 specifically. The NRRL 21882 strain of *A. flavus* does not produce aflatoxins, cyclopiazonic acid or known intermediates in the aflatoxin biosynthetic pathway. The provided honey bee data indicate that the proposed uses of afla-guard™ should not pose a hazard for insects (MRIDs 45884006, BPPD DER 07/16/2003b; 46196806, BPPD DER May 05, 2004c).

In summary, the justifications provided above indicate that the proposed uses of afla-guard™ should not pose an incremental hazard or risk greater than that which currently exists for freshwater fish, aquatic invertebrates, estuarine and marine animals, non-target plants and non-target insects (BPPD Review 05/06/2004d). Based on these acceptable justifications, the data required for these guidelines are waived for this proposed use of *Aspergillus flavus* NRRL 21882 on peanuts.

c. Endangered Species Assessment

Aspergillus flavus NRRL 21882 is a naturally occurring soil colonizer that does not produce aflatoxins, cyclopiazonic acid, or known intermediates in the aflatoxin biosynthesis pathway. Published literature searches conducted by the registrant for data waiver justifications found no relevant reports of adverse effects on wildlife. The product is intended for direct

application to soil once per year and submitted data show that, immediately post application, there is an increased concentration of *A. flavus* NRRL 21882, which diminishes the following spring. That is, the increased soil concentration is temporary. Other submitted literature citations show that natural populations of total *A. flavus* in soil can range from 0.5 to $>10^5$ CFU/g and can vary significantly (as much as ten-fold) within a single year in the same field. Further, the product is a granular formulation that minimizes the potential for drift, thus yielding an extremely low potential of *A. flavus* NRRL 21882 reaching bodies of water.

The combined evidence of literature citations provided by the registrant, product formulation, application, usage on peanuts, and a lack of published reports of adverse effects on wildlife indicate that exposure to afla-guard™ should have no measurable deleterious effects on endangered species. That is, there is a no "may effect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service (US FWS).

Table III.C.1.b: Eco-Toxicology Summary: Data Waivers

Guideline	Study	Status, Classification & Comments	Status
154-19 *885.4200	Fresh water fish testing	No hazards from *A. flavus* NRRL 21882 for freshwater fish, fresh water aquatic invertebrate, or to estuarine and marine animal are anticipated for this use. Low to no exposure of *A. flavus* NRRL 21882 to aquatic animals is expected for the intended use. An acceptable waiver rationale supports these findings.	Waived
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	No hazards from *A. flavus* NRRL 21882 for non-target plant species are anticipated for this use. Efficacy studies were conducted in greenhouse and field trials with *A. flavus* NRRL 21882 over > 10 years with no observed phytotoxic effects. An acceptable waiver rationale supports this finding.	Waived
885.4340	Non-target Insect Studies	No hazards from *A. flavus* NRRL 21882 for non-target insect species are anticipated for this use. An acceptable waiver rationale supports this finding.	Waived
None	Endangered Species Impact Assessment	The Agency performed an ESA assessment and determined that no adverse affects are expected to endangered species.	No labeling required.

*OPPTS Microbial Pesticide Harmonized Test Guideline Numbers.

Ecological Risks

Based on the studies and rationales for the data waivers discussed above, exposure and risk from the proposed use of *A. flavus* NRRL 21882, and its End-use Product, afla-guard™ on peanuts are expected to be minimal to non-target organisms including birds, mammalian wildlife, honey bee, other insects, freshwater fish and invertebrates, estuarine and marine animals, and

non-target terrestrial and aquatic plants **(Section III.C.** of this BRAD**).** The low application rates to soil once per growing season, low pesticide drift potential, the natural soil occurrence of the active ingredient, and its proposed use to displace aflatoxin-producing *A. flavus* strains, support this conclusion.

2. Environmental Assessment and Risk

Data citations provided by the registrant in support of their waiver justifications demonstrate that naturally occurring populations of total *A. flavus* have been reported to vary from 0.5 to $>10^5$ CFU/g and may vary significantly (greater than 10-fold) within a single field in a year. A three-year field study conducted by the USDA demonstrated that while the total *A. flavus* concentration increased with the application of *A. flavus* NRRL 21882, it declined by the spring of the following year. Similarly, a submitted unpublished study found there was an increase in the total population of *A. flavus* after application of afla-guard™, but, after years of testing, the total amount of *A. flavus* in the soil did not increase in the long term. Since the concentration of natural populations of *A. flavus* are shown to widely fluctuate, the increased concentration of *A. flavus* NRRL 21882 post application should not produce effects above those from natural *A. flavus* population fluctuations (BPPD Review 05/06/2004d).

The pesticide is applied at a low rate (approximately 1 gram or 0.002 pound active ingredient per acre). Thus, accumulation of *A. flavus* NRRL 21882 is not expected above existing population levels. Proposed displacement of aflatoxin-producing strains may decrease exposure and hazards posed by aflatoxin-producing *A. flavus*. 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 *A. flavus* NRRL 21882, or its end-use product afla-guard™, on peanut plants **(Section III.C.** of this BRAD**).**

No further testing for ecological effects is necessary for *A. flavus* NRRL 21882 for this proposed use on peanut plants. However, because the field trials were small scale, additional testing or research is required to satisfy concerns for product performance, or efficacy in reducing aflatoxin levels during large scale applications.

D. Efficacy Data - Product Performance [OPPTS 810.1000]

PR Notice 2002-1 lists aflatoxin as a public health hazard, for which product performance or efficacy data are required according to 40CFR158.202(i). To demonstrate that this pesticide may reduce aflatoxin-producing strains and does not increase *A. flavus* populations above background levels, the applicant provided product performance or efficacy data from multiple years of studies monitoring peanuts and its byproducts. Aflatoxins, some of the most potent human carcinogens, are the metabolites of concern that are produced by the target pest, aflatoxin-producing strains of *Aspergillus flavus*. As such, the Agency considers aflatoxins a public health hazard. In peanut-producing areas, when drought prevails, aflatoxin-producing strains of *Aspergillus flavus* are prominent.

Few alternatives, if any, exist to displace aflatoxin-producing *A. flavus* strains from peanuts and other crops. Costly irrigation, or treating peanuts by roasting, blanching or processing into peanut oil are among the methods used to decrease aflatoxins and aflatoxin-producing strains of *A. flavus* on peanuts. *A. flavus* NRRL 21882 is proposed to displace naturally occurring toxigenic *A. flavus* strains, and colonize the peanut during pegging or below ground (possibly by vector transmission) if conditions favorable to infection are present during the growing season - namely drought conditions without sufficient irrigation or presence of nematode or insect vectors that penetrate the peanut shell. The registrant has provided product performance data to demonstrate efficacy of the pesticide during 3 relevant small scale field trials in which the proposed EP was used at label rates. Aflatoxin in treated peanuts was decreased by 71 to 98% in comparison to untreated controls (BPPD DER, 05/05/2004a).

IV. PUBLIC INTEREST FINDING

The Agency believes use of *Aspergillus flavus NRRL 21882*} 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.

There is no pesticide registered to displace aflatoxin-producing strains of *A. flavus* from peanuts. Aflatoxins, potent human toxins and carcinogens that are considered public health hazards by the Agency, are the metabolites of concern produced by the target pest, aflatoxin-producing strains of *A. flavus*. Irrigation is one method of control for this public health hazard. However, irrigation is costly, and sometimes unavailable, in southern peanut growing states, e.g. Georgia, Alabama, Texas and Florida. Treatment of peanuts in these areas is likely to comprise less than 25% of the total US peanuts produced. The proposed pesticidal active ingredient, *Aspergillus flavus* NRRL 21882 is indigenous to the region, and has been shown to decrease levels of aflatoxins on peanuts in laboratory and small scale field trials **(Section III.D.** of this BRAD**).** No adverse effects have been reported by researchers of the USDA Agricultural Research Service, who have been engaged in research trials with this active ingredient.

Based on these rationales, the Agency has determined that *Aspergillus flavus* NRRL 21882 is likely to provide a cost effective biocontrol agent for reduction of aflatoxins in peanuts and its food/feed byproducts, and availability of the pesticide containing this active ingredient to growers 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 NRRL 21882 and its EP, afla-guard™ are eligible for a conditional registration because the proposed use of this active ingredient on peanuts is in the public interest. *Aspergillus flavus* NRRL 21882, when used as labeled, is not likely to pose an 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, and *A. flavus* NRRL 21882 (TGAI) and its End-use Product, afla-guard™ are eligible for conditional registrations for use on peanuts, in accordance with their label directions, if the registrant agrees to provide analyses of 5 production batches and efficacy studies from a large scale field trial. See **Section VI** of this document for actions to be taken by registrant.

2. Tolerance Reassessment

This is the first food use of this pesticide. No tolerance reassessment is required.

3. Ineligible Uses

Any other application of this pesticide, not in compliance with Agency requirements, will constitute a misuse.

4. CODEX Harmonization

There is 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 hazard to non-target organisms (plants, insects, aquatic freshwater estuarine and marine animals and wildlife), and to ground and surface water contamination through the proposed use of products containing *Aspergillus flavus* NRRL 21882 as discussed in this document as long as label directions are followed. No further mitigation measures are required at this time for dietary hazards, including those due to unintended exposure via drinking water. Appropriate PPE is required for pesticide handlers. These include long sleeved shirt, long pants, waterproof gloves, shoes, socks, goggles, and an appropriate dust/mist filtering respirator with the NIOSH prefix N–95, R-95 or P-95. The product label will also bear Environmental Hazards text to mitigate any potential risk as determined by reviewed data and use sites. This product may not be applied to aquatic or estuarine sites and residues of the pesticide may not be applied or disposed of in such a way that they are released into waterways.

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.

A discussion of the Endangered Species Assessment is found in **Section III.C.c.** of this BRAD**.** The Agency has made a no effect finding for the use *Aspergillus flavus* NRRL 21882 on endangered species. 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* NRRL 21882 must comply with the pesticide labeling requirements in existence when such products are registered.

1. TGAI /MP Product Labeling

The label must include appropriate statements to indicate that the registered product is a Technical Grade of the Active Ingredient product (TGAI) if the intent is to use the product to formulate end-use products (EP). PPE required for workers formulating the TGAI into the EP include: long sleeved shirt, long pants, waterproof gloves, shoes, socks, goggles and an appropriate dust/mist filtering respirator with the NIOSH prefix N–95, R-95 or P-95.

The following NPDES statement must be placed on the TGAI, *Aspergillus flavus* NRRL 21882, 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 Products containing *Aspergillus flavus* NRRL 21882 must comply with the pesticide labeling requirements in existence when such products are registered. For this proposed End-use Product, afla-guard™:

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 40CFR156.10 and other applicable notices, such as, and including the WPS labeling.

PPE for pesticide handlers (mixer/loader, applicators and post-application workers) include: long sleeved shirt, long pants, waterproof gloves, shoes, socks, goggles and an appropriate dust/mist filtering respirator with the NIOSH prefix N–95, R-95 or P-95. A Restricted-Entry Interval of 4 hours is required in which post application workers entering treated fields must wear the PPE required, as stated immediately above.

ii. Non-Worker Protection Standard

Only the agricultural crop, peanut plants, is addressed in this BRAD and is under the scope of the Worker Protection Standard, as noted immediately above.

iii. Other Precautionary Labeling

The Agency has examined the toxicological data base for *A. flavus* NRRL 21882 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 hazards associated with the proposed use. Additional

labeling may be required for other uses of products containing *A. flavus* NRRL 21882, on a case-by-case basis.

b. Environmental Hazards Labeling

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

Provided the following statement is placed in the environmental hazards statement, the risk of exposure to *A. flavus* NRRL 21882 is minimal to nonexistent to non-target aquatic organisms:

"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 labeling for pesticide products containing *A. flavus* NRRL 21882 must comply with current pesticide labeling requirements. The pesticide is to be applied as a granular formulation, by tractor mounted Gandy Box or similar appropriate equipment, at the pre-pegging phase of peanut plant growth (40-60 days after planting) at the rate of 20 pounds afla-guard™ (equivalent to 0.002 pounds active ingredient) per acre. Application after rain or when rain is expected will promote germination of the active ingredient.

D. Labeling

1. TGAI or Manufacturing Use Product

There is a separate technical grade of the active ingredient (TGAI) registered at this time for use as a manufacturing use product (MP). It must clearly state "For formulation into End-Use Products only".

Manufacturing Use Product Name: ***A. flavus* NRRL 21882**

Ingredient Statement:	w/w
Aspergillus flavus NRRL 21882.....	93.00 %*
Inert Ingredients	7.00 %
Total	100.00 %*

* viability of the End-use Product: minimum = 4.54×10^{11} CFU/lb TGAI
nominal = 7.71×10^{12} CFU/lb TGAI

Based on evaluation of the acute oral and pulmonary toxicity/infectivity exposure studies submitted to support registration of products containing *Aspergillus flavus* NRRL 21882, the signal word is "CAUTION". Signal words for other products containing this active ingredient will vary depending on toxicity/pathogenicity evaluations of those products.

2. End-Use Product Name: afla-guard™

Ingredient Statement:	w/w
Aspergillus flavus NRRL 21882	0.01 %*
Inert Ingredients	99.99 %
Total	100.00 %*

* viability of End-use Product: minimum = 4.54×10^{11} CFU/lb TGAI
nominal = 7.71×10^{12} CFU/lb TGAI

Based on the evaluation of the acute oral and pulmonary toxicity/infectivity exposure studies submitted for the active ingredient, the signal word is "CAUTION" for the End-Use Product, afla-guard™, containing 0.01% *Aspergillus flavus* NRRL 21882. Signal words for other End-use Products containing this active ingredient will vary on a case-by-case basis depending on 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 40CFR158.690(c) and 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 VI.a** of this BRAD.

1. Analyses of 5 batches are required at production and must include data relevant to detection, identification, enumeration and rejection limits of potential human pathogens (bacterial and fungal) and microbial contaminants, 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 *Aspergillus flavus* NRRL 21882 by taxonomy and VCG analysis.
(iii) analysis and quantification of metabolites and other unintentional ingredients, to include aflatoxins, cyclopiazonic acid and kojic acid;
(iv) identification and enumeration of potential human pathogens and other microbial contaminants;
(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 appropriately destroyed. The data from production batches (i thru vi, inclusive, listed above) are required as confirmatory data and must be submitted within the time frames noted in **Table VI.a of this BRAD** (within 30 months of the date of this conditional registration action).

While the registrant has provided demonstrable reduction of aflatoxins in peanuts during small scale field trials, similar efficacy studies have not been performed in large scale field trials. The small scale trials may not accurately reflect proliferation of *Aspergillus flavus* NRRL 21882, which facilitates competitive displacement of aflatoxin-producing fungal strains. Therefore, the Agency requires a large scale field trial and monitoring of treated and untreated peanuts for presence of visible fungus or mold as per the USDA visible inspection test, and for the regulatory levels of aflatoxins as required by the USDA and US FDA. Levels of aflatoxins in the pesticide itself are already regulated (see above). 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.

Table VI.a: Data required for *Aspergillus flavus* NRRL 21882 and afla-guard™

Guideline	Title of Study	Data required	Date due
*885.1300 151-12	Discussion of Formation of Unintentional Ingredients	5 batch analysis to include taxonomy and VCG analysis to identify *A.flavus* NRRL 21882, viability and storage stability data; identification and numeration of bacterial and fungal contaminants, analysis of metabolites including aflatoxins, cyclopiazonic acid and kojic acid.	Within 30 months of the conditional registration.
*885.1400 151-13	Analysis of Samples	Standard data requirement for production batches.	Within 30 months of the conditional registration
*885.1500 151-15	Certification of limits	Standard data requirement for production batches.	Within 30 months of the conditional registration
Non-guideline: required for public health hazards	Efficacy	Efficacy (product performance) data to demonstrate reduction of aflatoxins by *A. flavus* NRRL 21882 in a large scale field trial and to evaluate the visible effects and aflatoxins content of treated and untreated peanuts according to USDA and US FDA methods.	Within 30 months of the conditional registration

*OPPTS Harmonized Guidelines

VII. APPENDICES

APPENDIX A - USE SITES

Table VII.a lists the use sites for the product. The registrant must comply with the appropriate labeling requirements before releasing products containing *Aspergillus flavus* NRRL 21882 as the active ingredient for shipment.

Table VII.a: Use Site Conditional registration - *Aspergillus flavus* NRRL 21882 and afla-guard™

Peanut	**Official date registered:** May 28, 2004

APPENDIX B - BIBLIOGRAPHY

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May 05, 2004b. Avian Oral, (OPPTS 885.4050); DATA EVALUATION RECORD evaluating MRID 46240701. Hilary Hill and Zigfridas Vaituzis. US EPA/OPP/BPPD.

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