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DATA EVALUATION REPORT

Reviewed by:	Doug Gurian-Sherman, Ph. D., Plant Pathologist, 6/18/99 Biopesticides and Pollution Prevention Division
Secondary Reviewer:	Gail Tomimatsu, Ph.D., Plant Pathologist, 6/23/99 Biopesticides and Pollution Prevention Division

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STUDY TYPE:	Non-target Organism Environmental Safety Requirements, Waiver Request for Subdivision M, Guidelines: 154A-16 - 24
MRID NO:	None
TEST MATERIAL	*Aspergillus flavus* isolate AF36, provided by Sponsor
STUDY NO:	IR-4 PR No. 52B
SPONSOR:	IR-4 Project, Technology Center of New Jersey Rutgers University 681 U.S. Highway No. 1 South North Brunswick, NJ 08902-3390
TESTING FACILITY:	Southern Regional Research Center USDA/ARS P.O. Box 19687 New Orleans, LA 70179
TITLE OF REPORT:	Aspergillus flavus isolate AF36 Non-target Organism and Environmental Safety Information (Volume 6 of 7)
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STUDY COMPLETED: June 25, 1998

CONFIDENTIALITY CLAIMS: None.

CLASSIFICATION: Acceptable for EUP (see additional data requirements necessary for full registration)

Study Summary:

A. flavus is indigenous to the areas considered for use of AF36, where it is a common soil inhabitant. AF36 is a naturally occurring strain (MRID Nos. 43763401, 4390001). Use of AF36 will not change the amount of *A. flavus* in the environment (see appendix IV). The amount of organic matter added by the AF36 treated wheat seed (10 Lbs/A) is small compared to the amount of organic crop matter (appendix V) naturally colonized by *A. flavus*. *A. flavus* is commonly found in poultry feed, so exposure to poultry already occurs by this route. Assays of wheat seed from Arizona not treated with AF36 shows 100% *A. flavus* colonization in 1997 and 38% in 1998, therefore exposure from wheat fields already occurs (see appendix III). Use of AF36 in Arizona constitutes a minor use, and should be subject to regulatory relief. Lack of increased exposure applies to all of the required studies below.

Test Methods:

Most of the current study consists of summaries of data and methods are not included. Bird usage information by Chuck Youngker consists of anecdotal observations of birds and personal knowledge of cropping practices.

Occurrence of *A. flavus* on wheat seeds was determined from samples collected from wholesalers of Arizona wheat or from soil just after harvest. Control seed was from Hereford, TX. Wheat seeds were plated on Rose-Bengal agar (15-25 per plate), incubated at 31 ^{0}C for 5 to 7 days, and examined for *A. flavus* conidiophores.

Results Summary:

In addition to the above argument, avian oral toxicity/pathogenicity studies, (guideline number 154A-16), should be waived because bird exposure will not be increased. Observation of bird usage by cotton producer Chuck Youngker suggests that cotton fields are not preferred bird habitats and that birds are not attracted to them (appendix VII). Cotton fields are inhospitable after application in early June and provide little food.

even with the application of treated wheat seed at 10 lbs/A, especially since wheat fields with more wheat seed are available.

Poultry, which are highly susceptible to aspergillosis, are frequently exposed to fungi in enclosed in confinement houses. This exposure includes *Aspergillus* species. *A. fumigatus*, rather than *A. flavus*, is most commonly the cause of aspergillosis. Since pulmonary exposure will not be increased due to use of AF36, avian pulmonary toxicity/pathogenicity studies, guideline No. 154A-17, should be waived.

No arguments are presented to support waiver of wild mammal testing, guideline 154A-18. However, this study is included in the request (see title). Guideline study 154A-18 is conditionally required. Additional concerns will be addressed by pulmonary toxicity/pathogenicity studies being required for section 3 registration for human health effects.

A. flavus exposure of fish is not expected to be increased by the use of AF36 and fish are not reported to be susceptible to *A. flavus*, therefore freshwater fish toxicity/pathogenicity tests, guideline No. 154A-19, should be waived.

Since no adverse affects due to *A. flavus* have been reported for aquatic invertebrates, and exposure is expected to be minimal, test guidelines 154A-20 should be waived.

Waiver of estuarian and marine animal testing, guideline 154A-21, has not been specifically addressed but is included in the waiver request (see title). This study is typically only conditionally required.

Plant studies, guideline No. 154A-22 have been addressed in previous volume 6 titled "*Aspergillus flavus* Plant Studies - Request for Waiving the Requirement for Testing" (see appendix II). This volume is resubmitted.

A. flavus has been isolated from a number of insects (MRID No. 437634-03), but is not reported generally as an insect pathogen. therefore non-target insect testing, guideline 154A-23, should be waived.

Honey bees are not associated with cotton production, and "stone brood" caused by *A. flavus* is rare and of minor importance Therefore guideline 154A-24 should be waived.

Study Author's Conclusions

Based on the above considerations, all of the listed non-target organism tests should be waived.

Reviewer's Conclusion

Data submitted by Applicant and other published data agree that *A. flavus* is ubiquitous

in the Arizona desert. In addition to presence, levels of natural occurrence compared to treated fields must be considered in determining risk to susceptible organisms. Applicant presents summary data concerning soil populations of *A. flavus* prior to treatment and one year after application (page 43/70). These data suggest that populations of *A. flavus* are not increased by treatment one year after application compared to populations before treatment.

Applicant also presents data on the levels of propagules on treated and untreated crop matter and cottonseed in treated and untreated fields (page 45/70 and 46/70). The amounts of *A. flavus* on the crop was determined at maturity, when populations are typically highest. These data also indicate that populations of *A. flavus* are not significantly affected by application of the product to cotton fields.

However, while adequate to support the requested EUP, measurement of levels of total *A. flavus* on the crop at maturity and in the soil one year after application should continue for at least one season (depending on the results obtained). These measurements should continue because of the possible pathogenicity of the active ingredient, the heavy reliance of the Applicant on exposure and natural occurrence data, and especially the high degree of variability of the data, which is typical for measurements of this type.

Applicant has also argued elsewhere (Vol. 1, completed 12/8/98, reviewed below) that amounts of *A. flavus* in the soil are not increased during the growing season, nor at crop maturity. This data is supportive, but not conclusive, as explained below.

Applicant presents several arguments based on the relative biomass of the added product compared to the amount of biomass available to *A. flavus* already present in the environment, to suggest that the small amount of added AF36 will not increase overall *A. flavus* in the Arizona environment. These arguments cannot substitute for actual measurements of *A. flavus*. For example, it is not shown how good a growth substrate wheat is compared to other biomass. In addition, the product is prepared under conditions optimized for high inoculum production that may not typically occur in the field. Also, data are insufficient to demonstrate the amount of inoculum found on naturally occurring or agriculturally produced biomass for comparison with treated wheat seed.

Measurement of inoculum or propagule levels one year after application may not reflect higher transient amounts. For example, it is known that amounts of soil borne propagules increase dramatically in July and August. One year later, the nutritional substrate provided by the wheat seeds and other biomass may have been depleted, supporting lower amounts of AF36. Likewise, amounts of propagules on the crop may reflect saturation of the intrinsic carrying capacity of that biomass. Propagules above that amount produced by introduced inoculum may therefore not be reflected in these

measurements. Therefore, as detailed in the review below, propagule measurements performed at crop maturity must be conducted during the course of the EUP.

A. flavus is a known bird pathogen, causing invasive aspergillosis, often fatal when it occurs. Waiver of avian testing requirements is proposed based on exposure arguments discussed above and arguments that birds do not widely use cotton fields as habitat. Some of this information is of an anecdotal nature (e.g. as supplied by cotton producer Chuck Youngker), which is insufficient to base a waiver approval. Arguments that better food sources are available and that cotton fields are unsuitable habitats cannot substitute for actual data of bird usage of Arizona cotton fields. For example, while cotton is heavily managed, which Applicant argues would dissuade the presence of birds, there are still periods where there is little human activity in the fields. Cultivation, etc., may prevent or disrupt nesting, but may not prevent foraging by birds nesting elsewhere. Also, while humidity may be high, some birds may be attracted to the water supplied by irrigation. Actual bird census data, reviewed below, reveals a substantial presence of birds in Arizona cotton fields.

Applicant also points out that oral exposure is not noted in the literature as the usual route of infection of birds. However, oral infectivity cannot be ruled out, especially in wild species. Many studies do not examine route of exposure and assume that infection is respiratory due to infection of respiratory tissues.

Feeding is an important likely route of exposure for birds eating treated wheat seed. Therefore, oral avian toxicity/pathogenicity studies must be performed on quail before full registration can be approved.

Arguments for waiving avian pulmonary testing are also based on natural exposure. In addition, Applicant argues that poultry are susceptible in containment houses, where exposure is high. However, the infectivity in wild populations may not be well documented. As noted above and addressed below, transient levels of AF36 relative to untreated fields are currently not known with confidence. Such measurements have a high level of variability and may vary from year to year. In addition, nothing is known about the pathogenicity of AF36, and Applicant's isolate of AF36 in particular, which might be more or less virulent than other strains. Therefore, avian pulmonary tests must be performed on quail and preferably a second test species. However, since preliminary indications (see review below) suggest that transient levels of AF36 will not be significantly increased with treatment, these tests are required for full registration and may be performed concurrently with the EUP.

Concerns about freshwater and marine aquatic invertebrates and fish are adequately addressed by Applicant's arguments, and by the lack of exposure due to the location of application. Therefore, test requirements for these organisms may be waived.

Non-target plant testing has been adequately addressed by a prior EUP review (Tomimatsu and Rose, Memorandum dated 24 April1996, DP barcode No. D224186). Exposure will be highest in the cotton fields where non-target plant concerns will not apply. Exposure beyond the field is not expected to increase to levels of concern for non-target plants. Therefore, this test requirement may be waived, and the resubmitted study "*Aspergillus flavus* Plant Studies - Request for Waiving the Requirement for Testing" (completed July 31, 1995), will not be further reviewed here.

Applicant argues that *A. flavus* infection of honey bees, causing "stone brood", is a rarely reported occurrence and is considered a pathogen only of weakened hives. Applicants also argue that honey bees are not associated with cotton production. However, literature indicates that while cotton is not likely to be a preferred food source for honey bees, they will forage in cotton flowers, especially if preferred sources are not available (see e.g. ref.1). In addition, other bees, such as solitary species, are important pollinators in Arizona, and also visit cotton (see reference). Pathogenicity of species other than honey bees is unclear, but must be considered a distinct possibility given honey bee pathogenicity. In addition, as noted above, transient exposure considerations are currently unresolved, and the pathogenicity of AF36 is unknown. Therefore honey bee hive testing must be performed prior to full registration. Alternatively, Applicant may propose other local species of bee larva as an alternative for honey bees.

Applicant's arguments and data concerning other non-target insects are adequately addressed, and therefore other non-target insect testing may be waived.

Arguments or data supporting waiver of marine organism and wild mammal testing have not been addressed. However, Applicant has included the guideline numbers for these tests in the title of this study. Therefore, it is assumed that waiver of these tests is desired. Wild mammal testing has been partially satisfied by prior oral toxicity studies, and will be adequately addressed by required human safety tests for pulmonary toxicity/pathogenicity. Exposure to marine organisms is not expected to occur with this use of AF36. Therefore, these studies may be waived. However, Applicant should formally request waiver of these studies. 6 In addition, the methods for assaying environmental levels AF36 and *A. flavus* should be submitted where this has not already been done.

Reference:

D. Eisikowitch and G. M. Loper (1984) Some aspects of flower biology and bee activity on hybrid cotton in Arizona, USA. J. Apicultural Res. 23(4), 243-248

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R141778

Chemical: Aspergillus flavus 36 colonized wheat seed

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