US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Metarhizium anisopliae strain F52 (PC Code 029056)

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U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Metarhizium anisopliae strain F52
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I. ABSTRACT

Metarhizium anisopliae strain F52 is a microbial pesticide. TAE-001 Technical Bioinsecticide contains living *Metarhizium anisopliae* strain F52 as the active ingredient. *Metarhizium anisopliae* strain F52 is an deuteromycetous fungus with a host range primarily affecting coleopterans of the families Elateridae and Curculionidae, although other insect groups are known to be within the host range of this pathogen. This species is known worldwide from insects and non-insect sites, such as soil, river sediments, associated with nematodes, and as a saprophyte on organic detritus.

Metarhizium anisopliae Strain F52 is a pathogenic fungus that infects insects that come in contact with it. Once the fungus spores attach to the surface of the insect, germinate and begin to grow, they then penetrate the exoskeleton of the insect and grow very rapidly inside the insect causing the insect to die. Other insects that come in contact with infected insects also become infected with the fungus.

II. OVERVIEW

A. Product Overview

- **Microbial Pesticide Name:** *Metarhizium anisopliae* strain F52
- Trade Names: TAE-001 Technical Bioinsecticide; TAE-001 Granular Bioinsecticide;TAENURE GRANULAR BIOINSECTICIDE; TICK-EX™ EC TICK-EX™ G
- **OPP Chemical Code**: 029056
- **Basic Manufacturer:** Earth BioSciences

B. Use Profile

Type of Pesticide: Microbial Pesticide

Mechanism of action: *Metarhizium anisopliae* Strain F52 is a pathogenic fungus that infects insects that come in contact with it. Once the fungus spores attach to the surface of the insect, germinate and begin to grow, they then penetrate the exoskeleton of the insect and grow very rapidly inside the insect causing the insect to die. Other insects that come in contact with infected insects also become infected with the fungus.

Use Sites:

Terrestrial Non-Food
Greenhouse ornamental, nurseries, residential and institutional lawns, landscape perimeters

Target Pests for Active Ingredient:
Root Weevils, Flies, Gnats, Thrips, American Dog Tick, Blacklegged Tick, Brown Dog Tick, Lone Star Tick, Rocky Mountain Wood Tick, Rotund Tick, Grubs such as: Japanese Beetle Larvae, June Beetle Larvae, May Beetle Larvae, Oriental Beetle Larvae

Formulation Types Registered:

Type: Technical and End-use
Form:Technical at 97.6%; Granular at 2.0%; Emulsifiable Concentrate at 11%

Method and Rates of Application:

Equipment
Nozzle Sprayer

Timing
For Nurseries and Greenhouses use prior to or during planting. For Residential, Landscape Perimeters and Institutional Lawns apply as needed

Rates of Application:
25 to 50 grams per cubic foot of growing medium
1.0 to 3.0 lbs of Tick-EX G per 1000 square feet

Method of Application:
Spray; incorporate in growing media

C. Regulatory History

Earth BioSciences of New Haven, CT (formerly Taensa Company) submitted an application May 28, 1999 for registration of *Metarhizium anisopliae* strain F52 for non-food, indoor and greenhouse use. On June 14, 2002 the registrant (Taensa Company) submitted an

application for an additional Metarhizium anisopliae strain F52 granular product for treating additional sites and pests. This application indicated that TICK-EX™ G was to be used at residential and institutional lawns, and landscape perimeters to control ticks and grubs.

Metarhizium anisopliae strain F52 (a fungus) is an entomopathogen and will be used to control household and greenhouse insect pests. This is considered a non-food use and therefore no associated tolerance is requested at this time.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

Product Identity:

The agency has classified *Metarhizium anisopliae* strain F52 as a microbial pesticide. TAE-001 Technical Bioinsecticide contains living *Metarhizium anisopliae* strain F52 as the active ingredient. *Metarhizium anisopliae* strain F52 is a deuteromycetous fungus with a host range primarily affecting coleopterans of the families Elateridae and Curculionidae, although other insect groups are known to be within the host range of this pathogen. This species is known worldwide from insects and non-insect sites, such as soil, river sediments, associated with nematodes, and as a saprophyte on organic detritus.

Product chemistry data which support the registration of *Metarhizium anisopliae* strain F52 are summarized in Table 1.

Table 1. Physical and Chemical Properties for *Metarhizium anisopliae* strain F52

OPPTS GUIDELINE Number	STUDY	RESULT	MRID#
885.1100	Product Identity and Disclosure of Ingredients	The data submitted were not sufficient to warrant the conclusion that the method could be used to separate or identify F52 from other commonly encountered strains of *M. anisopliae*. An alternate laboratory will need to be sought to complete the comparisions of strains or another technique to accomplish the task of strain verification. The study was classified as **SUPPLEMENTAL**.	456963-01 448547-01
885.1200	Manufacturing Process	Additional data required. **SUPPLEMENTAL**	456963-02 448547-02 456963-03 448547-03
885.1300	Formation of Unintentional Ingredients	Additional data required. **SUPPLEMENTAL**	448447-04 456963-03
885.1400	Analysis of Samples	Approximately 48% of the extractable spores on the rice grains were recovered. The range of recovery was 40 to 59% and the viability of the recovered spores was highly consistent between batches. Study classified as **ACCEPTABLE.**	456963-04 456963-03 448447-06 448447-05
885.1500	Certification of Limits	Ingredient limits were 1.7 to 2.3 % for the a.i. and 97.7 to 98.3% for the matrix of the biopesticide. Both the nominal concentration of F52 spores and the proposed range were within the scope of what was observed in production of this agent. The study was classified as **ACCEPTABLE.**	456963-05 448447-02 448447-03 456963-03

B. Human Risk Assessment

There is a reasonable certainty that no harm will result from exposure to *Metarhizium anisopliae* strain F52. This includes all anticipated exposures which there is reliable information.

1. Human Toxicity Assessment

a. Acute Toxicity

All mammalian toxicology data requirements to support registration have been fulfilled by either submitted studies which adequately satisfy the data requirements or acceptable waivers. The acute oral, acute pulmonary toxicity/pathogenicity, acute dermal irritation and acute eye irritation studies resulted in Toxicity Category III classifications. The acute inhalation and primary dermal studies were waived based on the fact that there is little exposure and the end use products are mostly for greenhouse uses.

Table 2. Toxicity Data Requirements

OPPTS GUIDELINE NUMBER	STUDY	RESULT	MRID#
885.3050	Acute Oral Toxicity/ Pathogenicity	The oral LD_{50} of TAE-001 oil in rats was greater than 5000mg/kg. Toxicity Category IV. **ACCEPTABLE**	448447-09 457788-03
885.3100	Acute Dermal Toxicity/ Pathogenicity	LD_{50} of *Metarhizium anisopliae* strain F52 in rats is greater than 2000 mg/kg. **ACCEPTABLE**, Toxicity Category III	448447-10
885.3150	Acute Pulmonary Toxicity/ Pathogenicity	The LD50 value is known to be greater than 1.17 x 108 cfu/animal. **ACCEPTABLE**, Toxicity Category III	448447-11

OPPTS GUIDELINE NUMBER	STUDY	RESULT	MRID#
885.3200	Acute Intraperitoneal Toxicity/Pathogenicity	*Metarhizium anisopliae* strain F52 does not appear to be pathogenic in rats when dosed at 1x 10^7 cfu/animal **ACCEPTABLE** Toxicity Category III	448447-12
870.1300	Acute Inhalation (End-Use Product WP)		Waived
870.2400	Primary Eye Irritation	The LC_{50} of *Metarhizium anisopliae* strain F52 cannot be determined due to single dosing. **Supplemental**	448447-13
870.2500	Primary Dermal Irritation		Waived
870.2600	Delayed Contact Hypersensitivity in Guinea Pigs (QST 713 Wettable Powder)	*Metarhizium anisopliae*, Strain F52 was not a dermal sensitizer when induce and challenged at 2.37x 10^9 CFU. **ACCEPTABLE**	448447-15

b. Subchronic Toxicity and Chronic Toxicity

Subchronic and chronic toxicity were not required because survival, replication, infectivity, toxicity, or persistence of the microbial agent was not observed in the test animals treated in the acute oral infectivity test.

c. 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 hormone 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 has no information to suggest that *Metarhizium anisopliae* strain F52 has an effect on the immune and endocrine systems. No specific tests have been conducted with *Metarhizium anisopliae* strain F52 to determine such effects. However, as is expected from a non-pathogenic microorganism, the submitted toxicity/pathogenicity studies in rodents indicated that following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient. Therefore, it is unlikely that this organism would have estrogenic or endocrine effects because it is practically non-toxic to mammals.

When the appropriate screening and/or testing protocols being considered under the Agency's Endocrine Disruptor Screening Program have been developed, *Metarhizium anisopliae* strain F52 may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption. Based on the weight of the evidence of available data, no endocrine system-related effects have been identified for *Metarhizium anisopliae* strain F52.

2. Dose Response Assessment

No toxicological endpoints are identified.

3. Aggregate Exposure and Risk Characterization

a. Dietary

i. Food

In the absence of any toxicological endpoints, risk from the consumption of residues is not expected for the general population including infants and children. Further, this is a non-food use product; any dietary exposure would be inadvertent.

ii. Drinking Water

Metarhizium anisopliae is a deuteromycetous fungus that is known worldwide from

insects and non-insect sites, such as soil, river sediments, associated with nematodes, and as a saprophyte on organic detritus. Because of the use sites and amount of product that will be applied, it is not likely that use of this product will result in a significant increase in fungal spore exposure in drinking water. There is a low likelihood that they would survive passage through the soil to reach underground water. Even if the fungal spores were to reach underground water, it is highly unlikely that the spores would survive municipal water treatment. Therefore, it is likely there will not be an increase of *Metarhizium anisopliae* strain F52 in drinking water. In the absence of any toxicological endpoints, there are no concerns of *Metarhiaium anisopliae* contaminating drinking water.

b. Other Non-occupational Exposure

Deuteromycetous fungi are naturally occurring, and because of the use pattern and amount being applied, it is not likely that there will be a significant increase in potential exposure. The deutermomycetous fungi have a host range primarily affecting coleopterans of the families Elteridae and Curculionidae. No pathogenicity to mammals was observed in the submitted data. Therefore, even if there was an increase in exposure, there should not be any increase in potential human health risk.

4. Occupational, Residential, School and Day care Exposure and Risk Characterization

Children may be exposed to this product on treated ornamentals, turf and landscapes. The lack of mammalian toxicity at high levels of exposure to *Metarhizium anisopliae* demonstrates the safety of the product at levels well above maximum possible exposure levels anticipated. The potential for occupational and residential risk from exposure to lawns and ornamentals is expected to be minimal based on the submitted acute toxicity data.

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

There have been no confirmed reports of immediate or delayed allergic reactions to *Metarhizium anisopliae* strain F52 based on a review of published medical literature.

Based on the acute toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Metarhizium anisopliae*

strain F52. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because, as discussed in Unit B. Human Risk Assessment, *Metarhizium anisopliae* strain F52 is practically non-toxic to mammals and under reasonably foreseeable circumstances it does not pose a risk.

FFDCA section 408 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 pre- and post-natal toxicity and the completeness of the database, unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety) are often referred to as uncertainty (safety) factors. In this instance, the Agency believes there is reliable data to support the conclusion that *Metarhizium anisopliae* strain F52 is practically non-toxic to mammals, including infants and children, and, thus, there are no threshold effects; therefore, EPA has not used a margin of exposure (safety) approach to assess the safety of *Metarhizium anisopliae* strain F52 . As a result, the provision requiring an additional margin of exposure (safety) does not apply.

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

Because no toxic endpoints for mammals have been identified, and because no toxic effects have been reported from limited human exposure, no toxicity or pathogenicity is expected from aggregate exposure of the public via inhalation, dermal, and oral routes of exposure. Worker exposure via inhalation and dermal routes will be minimized by the use of personal protective equipment.

Based on the available information, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Metarhizium anisopliae* strain F52. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because *Metarhizium anisopliae* strain F52 shows no evidence of toxicity or infectivity in any organisms tested in support of this registration.

7. Cumulative Effects

Metarhizium anisopliae strain F52 is practically non-toxic to mammals. No mechanism of toxicity in mammals has been identified for this organism. Therefore no cumulative effect with other related organisms is anticipated.

C. Environmental Assessment

1. Ecological Toxicity

All non-target insect studies submitted to the Agency in support of *Metarhizium anisopliae* strain F52 registration demonstrated no adverse effects under the test conditions.

The Agency has performed an environmental risk assessment and determined that the proposed uses of *Metarhizium anisopliae* Strain F52 Biological Insecticide will have no adverse effects to avian species, wild mammals and terrestrial and aquatic plant species from residential outdoor and institutional premise uses of the product. In light of laboratory studies reporting toxicity and pathogenicity to immature aquatic vertebrate and invertebrate species, additional hazard assessment needs to be performed prior to any registration for aquatic applications. Freshwater and estuarine/marine fish, invertebrates and aquatic insects will not be affected by the quantities entering the aquatic environment from incidental drift and runoff from terrestrial uses. Submitted studies show that Strain F52 poses no hazard to lady beetles, green lacewings, parasitic wasps, honey bee larvae, honey bee adults and earthworms. Because *M. anisopliae* F52 uses are limited to those uses previously listed in this document, there also is no "may affect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

Registration for use sites other than residential outdoor premises will require additional hazard assessments for non-target insects and aquatic wildlife. Addition of playground, park land and campground use sites will require extensive endangered insect species reevaluations.

a. Background

Entomopathogenic fungi are promising alternatives to chemical insecticides. However, a major hurdle concerning the registration of these fungi as biological insecticides has been possible pathogenicity to a wide spectrum of insects and the toxicity of secreted metabolites. *Metarhizium anisopliae* is one of several common entomopathogenic fungi that can be used to control a variety of insect pests (particularly soil-dwelling and pasture pests). *M. anisopliae* has been reported to infect approximately 200 species of insects and other arthropods. In South America, *M. anisopliae* has been used against the spittle bug (*Mahanarva posticata*) and leafhoppers (Cercopidae). In South-East Asia, the fungus has

been used to control the coconut palm rhinoceros beetle (*Oryctes rhinoceros)* and another coconut pest, *Brontispa longissima*.. It has also been investigated for the control of the pasture cockchafer (*Aphodius tasmaniae)*, the beetle pasture pest (*Adoryphous couloni)*, the termite (*Nasutiterenes exitiosus)*, the sugar cane beetle (*Antitrogus parvulus)*, the pecan weevil (*Curculio caryae)*, the black vine weevil (*Otiorhynchus sulcatus)* and cockroaches. *M. anisopliae* has controlled many other insect pests in experimental trials, including Japanese beetle, black vine weevil, and mosquitoes. Sprayable formulations have been used to control meadow spittlebug on sugar cane and coffee leafminer and the froghopper (*Tomaspis saccharina*) in Trinidad and Grenada. *M. anisopliae* is highly pathogenic to many species of ticks, and is being considered as a microbial control agent for the management of ticks and Lyme disease. However, this fungus may also infect and kill beneficial organisms. In laboratory assays, the thrips predator *Orius insidiosis* showed a high rate of susceptibility to *M. anisopliae*. Genthner and Middaugh (1) reported that when developing embryos of the inland silverside fish, *Menidia beryllina*, were exposed to conidiospores of *M. anisopliae*, several adverse effects were observed in both embryos and newly-hatched fry. In a follow-up study designed to validate embryo tests for determining adverse effects of fungal pest control agents, Genthner et al. (2) presented data from a single experiment that suggested *M. anisopliae* was an invasive pathogen of embryos of the grass shrimp, *Palaemonetes pugio*.

M. anisopliae also produces a number of insecticidal toxins, including a number of destruxins. There is evidence to suggest that destruxins could play a role in determining host specificity. Specialized species like *M. album*, which is restricted to hemipteran insects, produce very little destruxin while generalist species like *M. anisopliae* var. *anisopliae* produce destruxin A, B and E, often in significant quantities. Destruxin E is active against many aphid species, as well as moth and fly larvae. Toxicity of *M. anisopliae* cultures was also examined by Genthner et al.(3) on several aquatic species. Toxicity was observed to mysids, developing grass shrimp (*Palaemonetes pugio)*, frog (*Xenopus laevis*) embryos; and juvenile mosquito fish (*Gambusia affinis*). However, adult female *G. affinis* survived a 24-h exposure and produced healthy broods. After three months, no mortalities or adverse effects were observed in adult *G. affinis* fed a diet partially composed of a freeze-dried *M. anisopliae* culture. Also, no lethal or teratogenic effect, or postponement of emergence of the embryos was observed in the teleostean fish.

The quantities of toxic secondary metabolites produced by these fungi *in vivo* are usually much less than those secreted in nutrient rich liquid media. Therefore use of mycoinsecticides is not expected to result in levels harmful to the environment. Likewise, the fungal spore dose required to produce an LD_{50} in susceptible insects is rather high, usually in the 10^6 - 10^7 CFU/mL range which is much higher than will occur from applications of *M. anisopliae* Strain F52 . In addition, variation in *M. anisopliae* strain specificity for selected insect species has also been widely reported (4). Entomopathogenic

fungi tend to adapt to certain species resulting in strains that are more infectious for some insect species than others.

The main ecological concern for *M. anisopliae* registration is effects on non-target invertebrates, principally beneficial insects. The data submitted and public literature support this contention. In a publication by M.S. Gottel et al. [Chap. 15 in Safety of Microbial Insecticides, M. Laird, L.A. Lacey and E.W. Davidson Eds., 1990. Safety to non-target invertebrates of fungal biocontrol agents, CRC Press, Boca Raton, FL], they concluded: "A review of the present knowledge on potential fungal control agents indicates that these organisms pose a minimal risk to non-target organisms. Indeed, when compared with chemical insecticides, fungal biocontrol agents offer, among other advantages, a method of control that has a very narrow host range, can usually be integrated with other biocontrol agents, may provide prolonged control by establishment and recycling within the habitat, and is also biodegradable." This article also concludes that: "In any case, the general consensus is that fungi do pose inherent, albeit minimal risks and therefore should be regulated in some manner. Consequently, registration is of paramount importance. Most guidelines for registration of entomopathogenic fungi require laboratory testing for infectivity to non-target invertebrates. However, limitations of the present knowledge of fungal specificity and how it relates to epizootiology make it impossible to extrapolate such data to the field situation. Nevertheless, limited laboratory infectivity studies with the formulated product against non-target invertebrates may identify potential hazards that should be addressed during field trials."

b. Non-target Organism Testing with *Metarhizium anisopliae* strain F52

The results of the non-target effects studies are presented here in both tabular (Table 3) and more detailed descriptive format. The complete review record of the submitted data can be found in the individual Data Evaluation Reports (DERs).

Table 3. Tabular Summary of Non-Target Organism Testing:

Guideline Number	Study	Result	MRID Number
885.4050	Avian Oral Toxicity Pathogenicity Study	The avian oral LD_{50} is > 5000 mg/kg body weight in a 30 day maximum hazard dose test. *Metarhizium anisopliae* Strain F-52 is considered non-pathogenic and non-toxic to bobwhite quail, an indicator avian hazard test species. The study is **supplemental** and upgradeable to acceptable provided the viability and stability of *M. anisopliae* in corn oil is determined and reported.	448447-19
885.4200	Freshwater Fish Testing	The 30-day LC50 for rainbow trout exposed to *M. anisopliae* was >53 mg/L, the highest concentration tested. All fish appeared normal and there were no mortalities in all treatment groups. The study is rated **supplemental** since a maximum hazard dose was not obtained and laboratory induced pathological changes in fish (silverside) embryos and fry has been reported in the literature.	448447-21
885.4240	Aquatic invertebrate testing	A 21-Day Life-Cycle Toxicity and Pathogenicity Test with *Daphnia magna* showed an EC_{50} of 1.19×10^9 CFU *M. anisopliae*/L. No adverse effects were noted at the EEC from direct application to aquatic environments. **Acceptable**.	448447-20
885.4150	Wild mammal Risk Assessment	The mammalian toxicity/pathogenicity data indicate no adverse effects in rodent testing at the maximum hazard dose. Therefore no further wild mammal testing is required.	N/A
885.4300	Terrestrial Plant Risk Assessment	*Metarhizium anisopliae* is generally known to be non-pathogenic to plants. No plant testing is required.	N/A
885.4300	Aquatic plants: Algae Growth Inhibition Test	Algal growth not affected as observed in growth recovery phase. This is not a required study. **Supplemental**.	448447-28
885.4340	Green lacewing dietary study	The *M. anisopliae* F52 LC50 for green lacewings is >600 ppm (4.2×10^7 CFU/g), the highest concentration tested. *M. anisopliae* strain F52 is not expected to pose a hazard to green lacewings in the field. **Acceptable**.	448447-22

Guideline Number	Study	Result	MRID Number
885.4340	Lady beetle dietary study	The *M. anisopliae* F52 LC50 for lady beetles is >600 ppm (4.2×10^7 CFU/mL), the highest concentration tested. Although not statistically significant, there was a slight increase in mortalities at 600 ppm. However, *M. anisopliae* F52 is not expected to pose a hazard to lady beetles because the EEC in the field is smaller than the 600 ppm test dose. **Acceptable**.	448447-23
885.4340	Parasitic wasp dietary study	The *M. anisopliae* F52 LC50 for *Nasonia vitripennis* is >600 ppm (4.2×10^7 CFU/mL), the highest concentration tested. *M. anisopliae* F52 is not expected to pose a hazard to parasitic wasps because the EEC in the field is smaller than the 600 ppm test dose. **Acceptable**.	448447-24
885.4340	Honey bee larva dietary study	The *M. anisopliae* F52 LC50 for honey bee larvae was >6,000 (cfu)/5 µL, the only concentration tested. There was no statistical difference in mortality between the negative control and *M. anisopliae* F52 treated groups. Results indicate that *M. anisopliae* F52 will not adversely affect honey bee emergence or survival. **Acceptable**.	448447-25
885.4340	Adult honey bee contact toxicity study	Results indicate that *M. anisopliae* F52 does not cause harmful effects or increased mortality to adult honey bees 26 days after being sprayed with field use rates of *M. anisopliae*. **Acceptable**.	448447-26
850.6200	Earthworm study	The 14-day LC50 is >1000 mg kg^{-1} dry soil, corresponding to > 7.00 x 10^7 CFU g^{-1} wet soil. There was no mortality or observed treatment related effects. **Supplemental**. (This study is not a guideline requirement for registration).	448447-27
885.4280	Estuarine and Marine Animal Risk Assessment	Estuarine/marine fish, invertebrates and aquatic insects will not be affected by the quantities entering the aquatic environment from incidental drift and runoff from terrestrial uses. For additional aquatic uses additional data are needed.	None

1) Avian Oral Testing, Tier I, USEPA OPPTS 885.4050 (MRID No. 448447-19)

A "Pathogenicity and Toxicity Study in the Northern Bobwhite" was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test. One aspect of the study not done according to GLP was that "[v]erification of the test concentration, stability and

homogeneity of the test substance in corn oil were not determined."

The LD_{50} dosage of *M. anisopliae* Strain F-52 was >3.5 x 10^8 cfu/g (5,000 mg/kg) of body weight per day for five days, the only concentration tested. The study indicates that *M. anisopliae* F52 did not cause mortalities in the birds and all birds showed "no signs of illness, abnormal behavior or appearance" in the negative control or in the treatment, attenuated control groups. The mean body weight and feed consumption were similar between the control and treatment groups. Gross necropsy of the control and treatment birds was unremarkable, except for the broken wing in the attenuated control bird. The study is supplemental and upgradable to acceptable provided the viability and stability of *M. anisopliae* Strain F52 in corn oil are determined and reported. The reviewed data indicate that TICK-EX$^{(TM)}$ when used as directed will not pose a hazard to avian wildlife.

2) Freshwater Fish Testing, Tier I, USEPA OPPTS 885.4200

A "Five-Concentration Toxicity and Pathogenicity Test With the Rainbow Trout (*Oncorhynchus mykiss*) " (MRID No. 448447-21) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test.

The 30-day LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to M. anisopliae was >53 mg/L, the highest concentration tested. Gross necropsies were performed on three surviving fish each from the dilution water control, each treatment concentration, and the attenuated control. None of the fish examined by gross necropsy exhibited signs of infection. Tissues of gills, intestines, and muscles of fish exposed to M. anisopliae appeared normal and were comparable to tissues of the negative control fish examined. M. anisopliae did not appear to be pathogenic to rainbow trout. All fish appeared normal and there were no mortalities in the negative control and in all the treatment groups. One out of the ten attenuated control fish died between day 7 and day 14. The lack of hazard at 2X EEC concentration for direct application to water indicates that no hazard to freshwater fish is expected from terrestrial application at the label use rates. However, the study is graded supplemental since a maximum hazard dose was not obtained, and laboratory induced pathological changes in fish (silverside) embryos and fry (not adult fish) have been reported in the literature.

3) Freshwater Aquatic Invertebrate Testing, Tier I, USEPA OPPTS 885.4240

A 21-Day Life-Cycle Toxicity and Pathogenicity Test With The Cladoceran (*Daphnia magna)*" (MRID No. :448447-20) was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of

Pesticide Programs in 40 CFR Part 160 with certain exceptions that did not affect the integrity of the test.

Mortality

Toxicity of the TGAI, *M. anisopliae* F52 to *Daphnia magna* was determined in a 21-day life-cycle study under static renewal conditions. Nominal concentrations of the test substance ranged from 2.5 mg/L (1.75×10^8 CFU *M. anisopliae*/L) to 40 mg/L (2.8×10^9 CFU *M. anisopliae*/L). Based on the probit method (Stephan, U.S. EPA), and the mortality data, the 21-day EC_{50} was calculated as 17 mg/L (1.19×10^9 CFU *M. anisopliae* /L). Mean percentage survival of daphnids after 21 days was 90% in the negative control and all surviving daphnids appeared normal and healthy. Survival in the attenuated control was 90%. Percentage survival in the *M. anisopliae* treatment groups decreased proportionately to increasing concentrations (nominal) of the test substance: 90%, 85%, 75%, 40% and 25% survival was reported in the 2.5, 5.0, 10, 20 and 40 mg/L treatments, respectively. The 95% confidence limits were 12 and 28 mg/L (8.4×10^8 and 1.96×10^9 CFU *M. anisopliae*/L) and the slope of the concentration response curve was 1.8. The NOEL and the lowest observed effect concentration were 5.0 mg/L (3.5×10^8 CFU *M.anisopliae*/L) and 10 mg/L (7.00×10^8 CFU *M. anisopliae*/L) respectively.

Reproduction

An average of 4.74 and 3.23 young per reproductive day were produced by adult daphnids in the negative and attenuated controls, respectively. Averages of 4.32, 4.45 and 3.12 young per reproductive day were produced by adult daphnids in the 2.5, 5.0 and 10.0 mg/L treatments, respectively. An average of less than 1 neonate per reproductive day was produced in the 20.0 and 40.0 mg/L treatment groups. The Dunnett's test showed that reproduction was significantly reduced in the attenuated control and in the 10 mg/L treatment group ($p \leq 0.05$). The NOEC for reproduction was 5.0 mg/L (3.5×10^8 CFU/L). The reduced reproduction rate in the attenuated control indicates that it was due to factors other than pathogenicity.

Growth Rate

Daphnids in the negative and attenuated control groups averaged 4.11 and 4.03 mm in length, and 0.537 and 0.448 mg of dry weight, respectively. Daphnids in the treatment solutions containing $\leq$ 5.0 mg/L had body lengths and body weights comparable to the negative control. Both length and dry weight were reduced significantly in the attenuated control and in the group exposed to 10 mg/L treatment compared to the negative control based on a Dunnett's test ($p \leq 0.05$). The NOEC for growth was 5.0 mg/L (3.5×10^8 CFU/L). The reduced growth rate in the attenuated control indicates that it was due to factors other than pathogenicity.

Conclusions

The study is rated as Acceptable. Because there were significant reductions in reproduction and growth in the attenuated control the mortality observed in all *M. anisopliae*-treatment groups proportional to the concentration tested could be attributed to a heat stable, or extracellular toxin or to the suspended particulate matter noted in the test solutions. Although toxicity was noted at the highest concentrations tested, no adverse effects were noted at the Expected Environmental Concentration (EEC) that would result from direct application to aquatic environments.

The lack of hazard at the EEC that would result from direct application to water indicates that no hazard to aquatic invertebrates is expected from drift or runoff after terrestrial application of TICK-EX(TM) at the label use rates.

4) Wild Mammal Risk Assessment, Tier I, USEPA OPPTS 885.4150

The wild mammal hazard assessment is being performed on the basis of rodent toxicity data prepared for human health risk assessment purposes and published literature reports. The standard mammalian toxicity test data submitted to the Agency indicate no adverse effects to rodents during the acute oral and itratracheal toxicity and pathogenicity testing at the maximum hazard dose. These data show a lack of toxicity to mammals from exposure to levels of M.anisopliae much higher than those encountered from the proposed registered uses of TICK-EX(TM). Therefore no further wild mammal testing is required.

5) Non-target Plant Risk Assessment, Tier I, USEPA OPPTS 885.4300

Terrestrial plants:
M. anisopliae is a naturally-occurring soil fungus whose level in the environment will not significantly increase with the use of TICK-EX(TM). *M. anisopliae* has not been known to cause pathogenicity in plants. *M. anisopliae* is not listed in the U.S. Department of Agriculture list of plant pathogens (Federal Plant Pest Act Regulations, 7CFR Part 330). In addition, a series of literature searches has been conducted to determine whether any adverse effects from *M. anisopliae* have been reported on plant species. There are no reports in the literature suggesting that *M. anisopliae* has detrimental effects on plants. In addition, efficacy testing with M. anisopliae (MRID No. 457237-01) has not indicated any pathogenicity, phytotoxicity, or any other adverse effects. Therefore, no hazard is expected to plants from the proposed uses of TICK-EX(TM). Therefore no further plant testing is required.
Aquatic plants:
A "96-hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum)* " (MRID No. 448447-28) was conducted. The Agency agrees with the study author's conclusions that "[b]ased on the growth observed in the recovery phase, the effects on algal growth were found to be algistatic, and not algicidal, at the concentrations tested." The study is rated

supplemental because living *M. anisopliae* cells in the test system compete for algal nutrients and can give the appearance of false positive (detrimental) effects on the algae. In addition, the actual measured concentrations of M. anisopliae were low. The plate counts were not highly variable within each treatment but differed greatly from the nominal concentration because the material did not suspend well. This was not a required study.

6) Non-target Insect Studies, Tier I, USEPA OPPTS 885.4340

The non-target insect studies were conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160 except the diets were not analyzed to verify concentration, homogeneity or stability of the test substance in the carrier.

Background:
Metarhizium anisopliae is an insect pathogenic fungus. The insect host range and the specificity of various *M. anisopliae* strains for particular insect species is reviewed above at the beginning of this Environmental Effects section. In order to ascertain the pathogenicity of *M. anisopliae* Strain F52 to beneficial insects, the applicant submitted non-target insect tests conducted at nominal concentrations including: 1. Dietary pathogenicity and toxicity of green lacewings (MRID No. 448447-22); 2. Dietary pathogenicity and toxicity of lady beetles (MRID No. 448447-23); 3. Dietary pathogenicity and toxicity of a parasitic Hymenoptera (MRID No. 448447-24); 4. Dietary effects on honey bee larvae (MRID No. 448447-25); and 5. A contact toxicity study on honey bees (MRID No. 448447-26). The most common route of insect infection is through direct cuticle penetration by a germinating spore. According to the Microbial Pesticide Test Guidelines OPPTS 885.4340 Non-target Insect Testing, Tier I, routes of exposure should simulate field conditions as much as possible. Although most entomopathogenic fungi control insects through external contact, various strains of *Metarhizium* have been shown to infect insects through ingestion or contact. The green lacewing, lady beetle, parasitic Hymenoptera, and honey bee larvae studies were conducted by feeding *Metarhizium anisopliae* strain F52 (technical grade active ingredient) mixed with the diet. These insects would be exposed to the *Metarhizium* via ingestion and contact as they are feeding. The adult honey bee study was conducted by direct spraying of the fungus onto the honey bees.

Conclusions:
All non-target insect studies submitted to the Agency in support of *M. anisopliae* strain F52 registration demonstrated no adverse effects under the test conditions. *M. anisopliae* strain F52 LC_{50} for green lacewings, lady beetles, and parasitic Hymenoptera is >600 ppm (4.2×10^7 CFU/g), the highest concentration tested. TICK-EX$^{(TM)}$ is, therefore, not expected to harm green lacewings, lady beetles, or parasitic Hymenoptera when used as directed. *Metarhizium anisopliae* strain F52 will also not adversely affect adult honey bees or larval

honey bee emergence or survival.

Green lacewing Dietary Study USEPA OPPTS 885.4380

"A Dietary Pathogenicity and Toxicity Study With Green Lacewing Larvae (*Chrysoperla carnea*)." was performed (MRID No.448447-22). Results from this study indicate that the LC_{50} of *M. anisopliae* strain F52 for green lacewings is >600 ppm (4.2×10^7 CFU/g), the highest concentration tested. No other adverse effects were observed. TICK-EX$^{(TM)}$ is, therefore, not expected to harm green lacewings when used as directed. EPA agrees with this conclusion.

As per the OPPTS Harmonized Microbial Testing Guidelines, the adult insect studies are to be of 30 days duration or until the negative control mortality reaches 20%. Total percentages of mortalities at the termination of the test (Day 12) were: 21% for the negative control, 23% for the attenuated control, 37% at 6.00 ppm, 27% at 60.0 ppm, and 33% at 600 ppm (Table 1). Differences in mortality were not significant (p<0.05). Surviving larvae from the control groups appeared normal throughout the test. Larvae from the treatment groups did not show apparent signs of toxicity. The study authors, therefore concluded that the observed mortality to green lacewings was not treatment related. EPA agrees with the conclusion.

Lady Beetle Dietary Study USEPA OPPTS 885.4380

"*Metarhizium anisopliae* Strain F52: A Dietary Pathogenicity and Toxicity Study With The Ladybird Beetle (*Hippodamia convergens*)" was conducted.(MRID No 448447-23). Results from this study indicate that the LC_{50} of *M. anisopliae* strain F52 for lady beetles is >600 ppm (4.2×10^7 CFU/mL), the highest concentration tested. No other adverse effects were observed. Although not statistically significant, there was a slight increase in mortalities at 600 ppm. This suggests there is a possibility of treatment-related effect. However, TICK-EX$^{(TM)}$ is not expected to harm lady beetles when used as directed because the possible effects were at >100 times the EEC.

As per the OPPTS Harmonized Microbial Testing Guidelines, the adult insect studies are to be of 30 days duration or until the negative control mortality reaches 20%. The total percentages mortalities at the termination of the test (Day 22) were: 21% for the negative control, 24% for the attenuated control, 17% at 6.00 ppm, 20% at 60.0 ppm, and 31% at 600 ppm. The noted differences in mortality were not significant (p<0.05). Surviving larvae from the control groups appeared normal throughout the test. The test substance, *M. anisopliae* strain F52, did not have a significant effect on mortality or insect behavior.

Parasitic Hymenoptera Dietary Study. USEPA OPPTS 885.4380

A "Dietary Pathogenicity and Toxicity Study With The Parasitic Hymenoptera (*Nasonia vitripennis*)" was submitted (MRID No. 448447-24). Results from this study indicate that the LC_{50} of *M. anisopliae* strain F52 for parasitic Hymenoptera is >600 ppm (4.2×10^7 CFU/g), the highest concentration tested. No other adverse effects were observed. Since the study did not show any harmful effects at >100 times the EEC, TICK-EX(TM) is not expected to pose any harm to parasitic Hymenoptera.

As per the OPPTS Harmonized Microbial Testing Guidelines, the adult insect studies are to be of 30 days duration or until the negative control mortality reaches 20%. The total percentages mortalities at the termination of the test (Day 26) were: 24% for the negative control, 51% for the attenuated control, 17% at 6.00 ppm, 17% at 60.0 ppm, and 20% at 600 ppm. If a control wasp that was near death when the study was terminated is considered as mortality, then the negative and attenuated control mortalities were 26%, and 52% respectively. The differences in mortality rates among the groups were not significant (p<0.05). Surviving *Nasonia vitripennis* appeared normal throughout the test except for occasional immobility observed and one near death in the 600 ppm treatment group.

Honey Bee Larva Dietary Study, USEPA OPPTS 885.4380

An "Evaluation of the Dietary Effect(s) of *Metarhizium anisopliae* strain F52 on Honey Bee Larvae (*Apis mellifera* L.)" was submitted for review (MRID No.448447-25). Two to three day old honey bee larvae were fed *Metarhizium anisopliae* strain F52. There were 20 bees in four replicates of the treatment and controls for a total of 80 *A. mellifera* tested per treatment. The treatment consisted of a nominal dose of 6,000 conidia spores (cfu)/5 µL. There was also a negative (30% sucrose solution in deionized water) and a positive (500 ppm mixture of potassium arsenate [Arsenic] in 30% sucrose solution) control treatment group.

Seven days after treatment the Arsenic control group sustained an average of 61.3% mortality. The negative control group sustained and average of 3.5% mortality. The treated group showed an average of 6.3% mortality. There was no unusual behavior by the emerged bees observed in any of the groups. There was not a statistical difference in mortality between the negative control and the treatment groups (p=0.05). Results indicate that *Metarhizium anisopliae* strain F52 does not adversely affect honey bee emergence or survival.

Honey Bee Contact Toxicity Study, USEPA OPPTS 885.4380

An "Evaluation of the Acute Contact Toxicity of *Metarhizium anisopliae* strain F52 on Adult Honey Bees (*Apis mellifera* L.)" (MRID No. 448447-26) was performed and submitted for review. A total of 225 bees at 25 bees per cage were sprayed with *M. anisopliae* F52 conidia in deionized water. Treatments were evaluated for mortality five hours post

treatment and daily thereafter for 26 days. Mean percentage mortality was calculated and compared via analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). There was not a statistically significant difference between percentage mortality of the control versus the treated group for any of the 26 days the test was conducted. Treated bees did not demonstrate any behavioral or morphological abnormalities. Results indicate that *Metarhizium anisopliae* strain F52 does not cause harmful effects or increased mortality when sprayed on adult honey bees.

7) Estuarine and Marine Animal Risk Assessment, Tier I, USEPA OPPTS 885.4280.

Genthner and Middaugh (1) (from the EPA marine research laboratory in Gulf Breeze, FL.) reported that when developing embryos of the inland silverside fish, *Menidia beryllina*, were exposed to conidiospores of *M. anisopliae*, several adverse effects were observed in both embryos and newly-hatched fry. In a follow-up study designed to validate embryo tests for determining adverse effects of fungal pest control agents, Genthner et al. (2) presented data from a single experiment that suggested *M. anisopliae* was an invasive pathogen of embryos of the grass shrimp, *Palaemonetes pugio*.

M. anisopliae also produces a number of insecticidal toxins, including a number of destruxins. There is evidence to suggest that destruxins could play a role in determining host specificity. Toxicity of *M. anisopliae* cultures was also examined by Genthner et al. (3) on several aquatic species. Toxicity was observed to mysids, developing grass shrimp (*Palaemonetes pugio)*, frog (*Xenopus laevis*) embryos; and juvenile mosquito fish (*Gambusia affinis*). However, adult female *G. affinis* surviving a 24-h exposure produced healthy broods. After three months, no mortalities or adverse effects were observed in adult *G. affinis* fed a diet partially composed of a freeze-dried *M. anisopliae* culture. Also, no lethal or teratogenic effect, or postponement of emergence of the embryos was observed in the teleostean fish.

The quantities of toxic secondary metabolites produced by these fungi *in vivo* are usually much less than those secreted in nutrient rich liquid media. Therefore use of TICK-EX$^{(TM)}$ is not expected to result in toxicity to aquatic wildlife. Likewise, the fungal spore dose required to produce a LD_{50} in susceptible species is rather high, usually in the 10^6 - 10^7 CFU/mL range. In addition, variation in *M. anisopliae* strain specificity for selected species has also been widely reported (4). Therefore, the proposed uses of TICK-EX$^{(TM)}$ are not expected to result in increased exposure of the estuarine/marine systems in the form of run-off. As a result, no hazard is expected to estuarine/marine wildlife from incidental drift and runoff from terrestrial uses of TICK-EX$^{(TM)}$. In light of the reported laboratory effects on immature estuarine wildlife reviewed above, additional hazard assessment needs to be performed prior to addition of direct aquatic uses of TICK-EX$^{(TM)}$.

8) Earthworm Subchronic Toxicity Study. USEPA OPPTS 850.6200

An "Earthworm (*Eisenia fetida*) toxicity study with *Metarhizium anisopliae* strain F52" (MRID No. 448447-27) was performed. The 14-day LC_{50} was >1000 mg/kg dry soil, corresponding to >671 mg/kg or >7.00 x 10^7 CFU/g wet soil. Adult earthworms with clitellum were exposed to the technical (a 5 concentration range) and heat-attenuated product mixed into an artificial soil, and a subset was exposed to soil only. Worms were examined at onset for burrowing activity and were removed from the substrate and closely examined (survival, appearance and behavior) at 7 and 14 days. At test termination (14 days) worms were also weighed. There was no mortality or observed treatment related effects and all worms appeared and behaved normally. Therefore, no hazard to earthworms is expected from the proposed uses of TICK-EX(TM).

The Agency has performed an environmental risk assessment and determined that the proposed uses of *Metarhizium anisopliae* Strain F52 Biological Insecticide will have no adverse effects on avian species, wild mammals and terrestrial and aquatic plant species from residential outdoor and institutional premise uses of the product. In light of laboratory studies reporting toxicity and pathogenicity to immature aquatic vertebrate and invertebrate species, additional hazard assessment needs to be performed prior to registration for aquatic applications. Freshwater and estuarine/marine fish, invertebrates and aquatic insects will not be affected by the quantities entering the aquatic environment from incidental drift and runoff from terrestrial uses. Submitted studies show that Strain F52 strain poses no hazard to lady beetles, green lacewings, parasitic wasps, honey bee larvae, honey bee adults and earthworms. Because *M. anisopliae* F52 uses are limited to outdoor residential premises, nurseries and greenhouses, and institutional lawns, there also is no "may affect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

Registration for use sites other than those listed above will require additional hazard assessments for non-target insects and aquatic wildlife. Addition of playground, park land and campground use sites will require extensive endangered insect species reevaluations.

2. Environmental Fate

Environmental fate studies are a Tier II requirement which was not triggered because the submitted non-target insect studies do not show a hazard from the proposed outdoor premise uses of *Metarhizium anisopliae* strain F52. Tier II testing is triggered only by a demonstrated hazard during Tier I non-target testing.

C. Hazard Assessment

Review of the non-target organism effects information in the public literature indicates that

the only ecological effects of some concern are to non-target insects. Natural epizootic caused by *M. anisopliae* occur among insect populations and the literature reports over 200 species of insects affected by this insect fungal pathogen. However, years of research have shown that *M. anisopliae* adapts to certain species resulting in strains that are more infectious for some insect species than others. The submitted studies show that *M. anisopliae* Strain F52 does not pose a hazard to the major beneficial insects tested, including honey bees. The possibility that some other non-target insects may also be infected cannot be ruled out.

The Agency has performed a hazard assessment and determined that the proposed uses of TICK-EX(TM) will have no adverse effects on avian species, wild mammal, freshwater and estuarine/marine fish and invertebrates, major beneficial insect species, including the honey bee, and terrestrial and aquatic plant species.

D. Environmental Risk Assessment

A risk assessment considers the hazard and the exposure in order to estimate potential risk. As stated in the Hazard Assessment above, the only adverse effects to non-target organisms would be to insects and fish. Representative insect species testing has indicated a general lack of adverse effects on terrestrial insects, especially at application rates. Some adverse effects were observed in test on aquatic animals (insects and fish).

Since there are reports of possible adverse effects on some immature aquatic animals, the product should bear the following precautionary statement under an Environmental Hazards heading on the label to avoid exposure to both freshwater and estuarine 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 by cleaning of equipment or disposal of equipment wash waters. Do not discharge into lakes, streams, ponds or public waterways." Elimination of direct aquatic application reduces exposure to aquatic organisms to only any runoff of spray drift. This is adequate to ensure no unreasonable adverse effects to non-target organisms.

E. Endangered Species Considerations

The reviewed studies and evidence in the public literature indicates that endangered terrestrial wildlife other than insect species would not normally be affected or infected by *M. anisopliae*. Also the product would be terrestrially applied precluding exposure to aquatic environments. Additionally the insecticides it is intended to replace have generally far greater side effects on non-target organisms. Such aquatic exposure could occur from runoff into adjacent water bodies. Since movement of *M. anisopliae* in soil into water bodies is expected to be negligible, the potential of aquatic organisms to be exposed to *M. anisopliae* F52 is minimal. In consideration of the public literature, it is plausible that *M. anisopliae*

may affect some endangered insect species, but TICK-EX(TM) would not generally be applied in areas where they may exist. Furthermore, it is not known whether *M. anisopliae* F52 affects or infects endangered insects nor is it appropriate to conduct such testing. Expansion of the registration to other use sites will require additional hazard assessments for endangered insect and aquatic wildlife. For instance, the addition of playgrounds, park land, campgrounds and agricultural use sites will require extensive endangered insect species reevaluations. Because the proposed uses of TICK-EX(TM) are not expected to result in exposure of endangered/threatened insect species habitats, there also is no "may effect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

F. Efficacy Data

Four studies on the efficacy of *M. anisopliae* on ticks were presented. Study one indicated the LD_{50} of *M. anisopliae* Strain F52 to *Ixodes ricinus* was $9.9x10^6$ conidia/mL. The LT_{50} for $1x10^8$ and $1x10^9$ spores/mL test suspensions were 14.8 and 11.2 days, respectively. Study two was a field study where *M. anisopliae* Strain F52 was applied in an oil-based formulation to residential properties at a rate of $2.3x10^{11}$ viable spores per 1,000 square feet. Preliminary results for ticks collected from drag sampling of lawns and woods in one Connecticut field study indicate 93.2% reduction of ticks in lawns and 67.1% reduction of ticks in woods treated with the fungus. Study three was a laboratory pathogenicity study of *M. anisopliae* (Strain MADA) to *Ixodes scapularis*. The LC_{50} was $1x10^7$ spores/mL. Tick mortality was positively related to spore concentration. Study four is an overview of the efficacy of *M. anisopliae* (TAE-001, 2.86% a.i) and other biopesticides, including *Beauveria bassiana* (Naturalis-O, 7.16% a.i.) and cinnamaldehyde against a tick relative, the two spotted spider mite, *Tetranychus urticae*. The overview documents the general consensus that $1x10^7$ to $1x10^8$ spores/mL are effective in reducing fecundity, egg hatchability and increasing mortality to ticks. However, a wide range in efficacy among the several strains of *M. anisopliae* was reported, thus additional testing is recommended to isolate and identify the most potent strains for biological control efforts. The registrant must submit the field efficacy data for the control of blacklegged ticks (*Ixodes scapularis*) when the study has been completed. This study must be submitted within one year from the date of this registration. Furthermore, because different families of ticks will behave differently and therefore be controlled differently, efficacy data must be developed for each specified public health pest. The level of control (pest population reduction) is not expected to be the same for control of a dog tick versus a deer tick, for example.

IV. Risk Management And Re/Registration Decision

Determination of Eligibility

Data submitted are sufficient for the conditional registration under Section 3(c)(7)(C) of FIFRA of *Metarhizium anisopliae* Strain F52 for the use patterns discussed in this document. The Agency will conditionally register this pesticide for a period of time reasonably sufficient for the generation and submission of additional studies that the Agency believes are required. Use of the pesticide during this period of conditional registration will not cause unreasonable adverse effects on the environment, and the use of this pesticide is deemed to be in the public interest because of the product's efficacy against ticks (a public health pest).

Regulatory Position

1. Conditional Registration

Section 3(c)(7)(C) of FIFRA provides for the conditional registration of new active ingredients if it is determined that 1) use of the pesticide during a defined period of time will not cause any unreasonable adverse effect on the environment; 2) use of the pesticide is in the public interest; and 3) that for the data that is lacking, a reasonable period of time sufficient for generation of the data has not elapsed since the Agency first imposed the data requirements.

To satisfy criterion (1) above, it is believed that this new microbial pesticide will not cause any unreasonable adverse effects on human health or the environment. Sufficient data are available to determine that Metarhizium anisopliae Strain has low toxicity to mammals and is not expected to be pathogenic in humans when the product is used in accordance with label instructions. Standard personal protective equipment are required to mitigate any risk to pesticide handlers and applicators. No significant risk is expected from the terrestrial ground application on approved sites of the end-use products to birds, fish, ladybird beetles, green lacewings, parasitic wasps and aquatic invertebrates. To satisfy criterion (2) *Metarhizium anisopliae Strain F52* is for use on residential lawns, institutional lawns, landscape perimeters greenhouses and nurseries. *Metarhizium anisopliae Strain F52* has shown some efficacy against tick species. According to the Agency's regulations on public interest findings (51FR No. 43), the public interest finding is presumed when a product is for use against a public health pest; therefore, the public interest finding is satisfied. (3) Finally, the outstanding data were not identified in the new active ingredient screening process and a reasonable period for its generation has not yet passed.

The data have been reviewed and EPA has determined that a section 3(c)(7)(C) conditional

registration that is limited in duration is appropriate in this situation. Data requirements for granting the subject time-limited registrations for terrestrial ground outdoor use under section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been met. EPA has determined that the use of this product will cause no unreasonable adverse effects to the environment if it is used pursuant to the following limitations.

Standard personal protective equipment are required to mitigate any risk to pesticide handlers and applicators.

Risks to endangered species of insects are mitigated by prohibiting use on or near camp sites, playgrounds, shadehouses or areas close to aquatic environments.

Confirmatory data will be submitted for freshwater fish.

The registrant must submit an efficacy study sufficiently addressing the deficiencies as outlined in section III.E.

The data for Product Identity, Manufacturing Process, Formation of unintentional ingredients guidelines require additional information. Detailed requirements are described in Confidential Business Appendix.

Before this manufacturing process can be changed, adequate storage stability data must be provided to the Agency and approved.

Data listed in Table 4 are required to be completed and submitted within specified time frames, as elaborated in Section V, Actions Required of Registrant.

These conditional registrations will automatically expire in two (2) years if the outstanding information is not adequately addressed.

There are no food uses associated the *Metarhizium anisopliae* strain F52 products being registered.

CODEX Harmonization

There are no CODEX values for *Metarhizium anisopliae* strain F52 . The products being registered are not for food use.

Risk Mitigation

Endangered Species Statement

DO NOT USE NEAR SITES WHERE THREATENED OR ENDANGERED SPECIES MAY BE FOUND OR NEAR AQUATIC AREAS THAT MAY CONTAIN THREATENED OR ENDANGERED SPECIES.

Labeling Rational

Human Health Hazard (WPS and non-WPS)

Metarhizium anisopliae strain F52 products with commercial use sites are subject to the Worker Protection Standard. Because of the low toxicity of *Metarhizium anisopliae* strain F52, the Re-Entry Interval for uses within the scope of WPS is 4 hours. Precautionary statements and personal protective equipment, as specified below, are required based on the acute toxicity categories of this organism.

Environmental Hazard

Precautionary labeling is required as indicated below.

V. ACTIONS REQUIRED OF REGISTRANT

A. Precautionary Labeling

Metarhizium anisopliae strain F52 products must state the following under the heading "Precautionary Statements":

Personal Protective Equipment required for Applicators as well as other handlers are listed below:

Coveralls, waterproof gloves, shoes plus socks, dust-mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N, R, P or HE filter.

WPS labels must state the following under the heading "User Safety Recommendations."

Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.

Users should remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Users should remove PPE immediately after handling this product. If gloves are worn, wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

B. Environmental Hazards Labeling

Provided the following statement is placed into the environmental hazards statement, the risk of *Metarhizium anisopliae* strain F52 is minimal to nonexistent to non-target organisms including endangered species.

1. End-Use Product Environmental Hazards Labeling

"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 by cleaning of equipment or disposal of equipment washwaters. "

2. Manufacturing-Use Product Environmental Hazards Labeling

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public water unless this product is specifically identified and addressed in an NPDES permit. Do not discharge effluent containing this product to sewer systems without previously notifying the sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA."

3. Application Rate

It is the Agency's position that the labeling for the pesticide products containing *Metarhizium anisopliae* strain F52 as the active ingredient complies with the current pesticide labeling requirements. The Agency has not required a maximum number of applications per a season of this active ingredient.

C. Labeling

Some of the essential label requirements are highlighted below.

Signal word is "Caution," based on (toxicity category III). The product shall contain the following information:

- Product Name
- Ingredient Statement
- Registration Number

- "Keep Out of Reach of Children"
- Signal Word (CAUTION)
- First Aid Statement
- Personal Protective Equipment (PPE) Requirements
- Environmental Hazard Statement
- Storage and Disposal Statement
- Agricultural Use Requirements
- Non-Agricultural Use Requirements
- Directions for Use

TABLE 4: SUMMARY OF CONDITIONAL REGISTRATION DATA REQUIREMENTS

Data Required			
Guideline	**Title of Study**	**Data required**	**Date due**
*885.1100	Product Identity and Disclosure of Ingredients	Additional data are required to upgrade submitted data to acceptable	1 year from date of registration
151-11 *885.1200	Manufacturing Process	Additional data are required to upgrade submitted process, to acceptable.	1 year from date of registration
885.13	Formation of Unintentional Ingredients	Additional data are required to upgrade submitted data to acceptable	1 year from date of registration
885.42	Freshwater Fish Testing	This study must be repeated. The maximum hazard dose was not obtained in the submitted study.	1 year from date of registration
40CFR 158.202(i)	Product Performance Data	The registrant must submit the field efficacy data for the control of blacklegged ticks (*Ixodes scapularis*). Efficacy data must be developed for each specified public health pest.	1 year from date of registration

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MRID: 44844700
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