Bacillus thuringiensis subspecies *israelensis* strain EG2215 (006476) Fact Sheet

I. Description of the Active Ingredient

- **Generic Name of the Active Ingredient:** Bacillus thuringiensis subspecies israelensis strain EG2215

- **Trade and Other Names:** BTI Technical Powder Bioinsecticide

- **OPP Chemical Code**: 006476

- **Year of Initial Registration:** 1998

- **Pesticide Type:** Microbial Insecticide

- **U.S. and Foreign Producers:**

  Ecogen, Inc.
  2005 Cabot Blvd. West
  Langhorne, PA 19047-3023

II. Use Sites, Target Pests, and Application Methods

- **Target Pests:** Mosquito larvae

- **Registered Uses:** Bacillus thuringiensis subspecies israelensis strain EG2215 is a manufacturing use product designed for production of end-use products for mosquito control. The end-use sites are for outdoor use only and include irrigation ditches, roadside ditches, flood water, standing ponds, woodland pools, snow melt pools, pastures, catch basins, storm water retention areas, tidal water, salt marshes, and rice fields.

- **Application Timing:** Application timing is not specified because the registered product is for manufacturing use only.

III. Science Findings

A. Microbe Description

The active ingredient for Ecogen BTI Technical Powder Insecticide is identified as Bacillus thuringiensis subspecies israelensis strain EG2215. The microorganism produces five cry toxins with Dipteran activity: cry4A, cry4B, cry10A, cry11A, and cytIA.

B. History

The original strain isolate for EG2215 is ONR-60A (Goldberg and Margalit, 1997) which is identical to strain HD567 from the H. Dulmage USDA collection (Dulmage, Beegle, Barjac, Reich, Donaldson, and Krywienczyk, 1982). Samples can be obtained from the WHO Collaborating Centre for Entomopathogenic Bacillus, Institut Pasteur, Paris, France.

C. Taxonomy

All *Bacillus* species are rod-shaped, endospore-forming aerobic bacteria. Strain EG2215 consists of large sporangia (at least 1.0 by 3.0) and vegetative cells. Strain EG2215 contains spores that are ellipsoidal, paracentral, do not distend to the sides of the sporangium, and require oxygen for sporulation. When grown on NSM agar, the colonies were opaque, cream colored, round, flat, and with a dry smooth texture. The crystal is the portion of the bacteria that gives it insecticidal properties and identifies the bacteria as *Bacillus thuringiensis*. Flagella are assumed to be present because the cells are motile. The resident plasmids contained in strain EG2215 include: ~135-, 105-, 75-, 68-, 10.6-, 4.9-, 4.2-, and 3.3- megadaltons.

D. Toxicology

1. Toxicity/Pathogenicity Data

The acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, and acute intravenous toxicity/pathogenicity data requirements for *Bacillus thuringiensis* subspecies *israelensis* strain EG2215 were satisfied via the cite-all method. The Agency does not anticipate any toxicological or pathogenic problems with *Bacillus thuringiensis* subspecies *israelensis* strain EG2215. However, to confirm these

expected results, the Agency will require that the registrant perform an Intraperitoneal injection study as a condition of registration. The assay must be performed with mice using three doses (10^6, 10^7, 10^8 CFU per animal). If this test reveals significant mortality, the Agency may require additional data. All registrants of products with *Bacillus thuringiensis* are currently required to perform an Intraperitoneal assay. In addition, to qualify for an exemption from tolerance, Ecogen must follow the production control measures outlined in 40 CFR 180.1011 to demonstrate lack of contaminants.

2. Product Specific Data

Ecogen BTI Technical Powder Bioinsecticide, containing *Bacillus thuringiensis* subspecies *israelensis* strain EG2215 has been supported with data from other *Bt kurstaki* products. Ecogen has cited the manufacturing process for Condor OF, a product containing *Bt kurstaki*. To confirm the lack of potential irritation in the final technical product, the Agency will require that an eye irritation study (81-4) and a dermal irritation study (81-5) be completed as a condition of registration. Until these tests are completed, all products containing *Bt israelensis* strain EG2215 are considered Toxicity Category II for eye irritation and must be labeled as such

Acute Oral Toxicity/Pathogenicity - Rat (81-1)

This study was cited from the Ecogen product CutlassTM WP; MRID# 413342-01

CLASSIFICATION: ACCEPTABLE-TOX CATEGORY IV

Acute Inhalation Toxicity - Rat (81-3):

This study was cited from the Ecogen product CutlassTM WP; MRID# 413342-01

CLASSIFICATION: ACCEPTABLE-TOX CATEGORY IV

Primary Eye Irritation - Rabbit (152A-14):

This study was cited from the Ecogen product CutlassTM WP; MRID# 423608-01

CLASSIFICATION: ACCEPTABLE-TOX CATEGORY II (see above)

E. Human Health Effects

1. Risks Posed by Potential Residential, School or Day Care Exposure

Bacillus thuringiensis israelensis strain EG2215 is labeled as a manufacturing use product for formulation into end-use products that may have outdoor residential uses. However, the toxicology of the active ingredient indicates that there will be minimal to nonexistent nondietary risk to children present from use sites allowed in end-use products formulated from *Bt israelensis* strain EG2215. The toxicology of *Bt* subspecies *israelensis* is well established. Thirty years of widespread *Bacillus thuringiensis* use has produced no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal, and inhalation exposure to the microbial product.

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408 provides that EPA shall apply an additional tenfold 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. In this instance, based on all the available information, the Agency concludes that infants and children will consume only minimal, if any, residues of this microbial insecticide and that there is a finding of no toxicity. Thus, there are no threshold effects of concern and, as a result the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply.

The registrant is required to report any hypersensitivity reactions as a result of the product's use when and if they occur.

2. Effects on the Endocrine System

The active ingredient is a microorganism. The Agency has considered, among other relative factors, available information concerning whether *Bacillus thuringiensis*may have an effect in humans similar to an effect produced by a naturally occurring estrogen or other endocrine effects. No known toxins or metabolites of *Bacillus thuringiensis* have been identified to act as endocrine disrupters or

immunotoxicants. Therefore, adverse effects to the endocrine or immune systems are not expected.

3. Potential for the Transfer of the Pesticide to Drinking Water

Bacillus thuringiensis subspecies *israelensis* is formulated for use on mosquito arvae in aquatic habitats. Although this product will be labeled for direct application to water, end-use products formulated from *Bacillus thuringiensis* subspecies *israelensis* strain EG2215 must be labeled with the following statement "Do not apply directly to treated, finished drinking water reservoirs or drinking water receptacles." This labeling language should reduce the potential of this product being applied directly to drinking water.

Bt is ubiquitous in many soils throughout the world. *Bt* is not known as an aquatic bacterium, and therefore, is not expected to proliferate in aquatic habitats. Although the potential exists for some minimal amount of the applied *Bt* to enter ground water or other drinking water sources, the amount present would in all probability be undetectable or at least several orders of magnitude lower than those levels tested for safety. Also, drinking water is not screened for *Bt* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. The municipal treatment of drinking water would further reduce the possibility of exposure to *Bt* through drinking water. Therefore, the Agency considers the potential of significant transfer of *Bt israelensis* strain EG2215 to drinking water as minimal to nonexistent.

4. Aggregate Exposure From Multiple Routes Including Oral, Dermal, and Inhalation

Bacillus thuringiensis is a naturally occurring soil bacterium. Anyone coming in contact with the soil is likely to be exposed to this microorganism. Because the health risk is expected to be negligible for oral, dermal, and inhalation exposure routes, as previously indicated, aggregate exposure by these routes, from naturally-occurring populations in the soil and from the use of pesticidal products, should not pose a threat to human health.

To further mitigate risk due to inhalation, all end-use products containing *Bacillus thuringiensis* subspecies *israelensis* strain EG2215 will be required to include the following statement on the label (this statement is required for all end-use *Bt* products):

"As a general precaution when exposed to potentially high concentrations of living microbial products such as this, all mixer/loaders and applicators not enclosed in

cabs or aircraft must wear a dust/mist filtering respirator meeting NIOSH standards of at least N-95, R-95, or P-95."

F. Food Quality Protection Act Requirements

No unreasonable adverse effects to human health are expected from the use of *Bt israelensis* strain EG2215. No tolerance is needed since *Bt israelensis* strain EG2215 is covered by the exemption from the requirement of a tolerance per 40 CFR 180.1011. The Agency has considered this *Bt* isolate in light of the nine safety factors listed in the Food Quality Protection Act and made a determination of reasonable certainty of no harm. In short, the Agency has not identified any subchronic, chronic, immune, endocrine, or non-dietary cumulative exposure issues as they may affect infants and children or the general population.

G. Ecological Effects

The non-target data requirements for *Bt israelensis* strain EG2215 were bridged from data submitted for other *Bt israelensis* registrations. *Bt israelensis* strain EG2215 has been positively identified as a typical *Bt israelensis* and it is unlikely that the toxicology of this microorganism will differ from other strains of *Bt israelensis*. However, the Agency is concerned with the potential presence of heat labile exotoxin produced during the manufacturing process for all *Bt* active ingredients. For this reason, a *Daphnia* study (with a 10 day exposure period) will be required as a condition of registration for *Bt israelensis* strain EG2215. Should this test show significant lethality, a dose response *Daphnia* test must be performed to derive an LC_{50}. Pending the results of this assay, further non-target species testing may be required by the Agency.

A summary of *Bt israelensis* toxicity to non-target organisms is presented below:

Toxicity to Terrestrial Animals

Birds: mallard duck and bobwhite quail (154-16)

Cited studies indicate that *Bt israelensis* is not toxic or pathogenic to either of these species after acute or subacute testing.

Non-target insects: green lace wing larvae, parasitic hymenoptera, predaceous Coleoptera (154-23)

Cited studies indicate that *Bt israelensis* shows little to no toxicity in any of the tested species.

Non-target insects: honey bee (154-24)

Cited study demonstrates that *Bt israelensis* has minimal toxicity to honey bees.

Toxicity to Aquatic Animals

Freshwater fish: trout and bluegill (154-19)

Cited studies indicate no toxicity or pathogenicity associated with *Bt israelensis*.

Freshwater invertebrates: *Daphnia* (154-20)

Cited study indicates that *Bt israelensis* is moderately toxic to *Daphnia*. The use rates of all end use products formulated from *Bt israelensis* strain EG2215 will be evaluated prior to registration to determine the risk to non-target aquatic invertebrates.

Estuarine and marine animals: grass shrimp, sheepshead minnow, copepod (154-21)

Cited studies indicate no toxicity or pathogenicity in these species associated with *Bt israelensis.*

Toxicity to Non-target Plants

Since the Agency is not aware of any evidence that *Bacillus thuringiensis* causes any adverse effects to plants, the risk to non-target plants (including terrestrial, semi-aquatic, and aquatic species) is minimal.

Toxicity to Mammals

Based on the mammalian studies cited in the health effects assessment and the history of thirty years of safe *Bt* use, the risk to mammalian wildlife should be minimal to nonexistent. However, an Intraperitoneal injection study will be required as a condition of registration specifically for *Bacillus thuringiensis* strain EG2215. This test is part of the product characterization to demonstrate lack of mammalian toxicity in the Technical Grade of the Active Ingredient (TGAI).

Risk to Endangered Species

Based on the toxicity and exposure data for *Bacillus thuringiensis*, there should be no risk for endangered mammals, birds, plants, and noninsect aquatic species. All endangered/threatened insect species that are susceptible to the *Bacillus thuringiensis* subspecies *israelensis* endotoxins may be adversely affected if exposed.

H. Environmental Fate and Ground Water Effects

Exposure assessments on this type of microbial pesticide are not performed unless significant human health or ecological effects issues arise in the Tier I studies (40 CFR 158.690 (c)). Since Tier II studies were not triggered, there is no requirement for environmental fate data.

I. Efficacy Data

Bacillus thuringiensis strain EG2215 is registered as a manufacturing use product only. It will ultimately be used to formulate end use products for mosquito control. Since *Bt israelensis* strain EG2215 is a manufacturing use product and bears no label claims for end use and direct application for control of mosquitoes, efficacy data was not required. However, any end use products formulated from *Bt israelensis* strain EG2215 for control of mosquitoes must be registered separately and will be subject to efficacy data requirements.

IV. Labeling

. Endangered Species

Currently, the Agency is developing a program ("The Endangered Species Protection Program") to identify all pesticides whose use may cause adverse impacts on endangered and threatened species and to implement mitigation measures that will eliminate the adverse impacts. The program would require use restrictions to protect endangered and threatened species at the county level. Consultations with the Fish and Wildlife Service may be necessary to assess risks to newly listed species or from proposed new uses. In the future, the Agency plans to publish a description of the Endangered Species Program in the Federal Register and have available voluntary county-specific bulletins. Because the Agency is taking this approach for protecting endangered and threatened species, it is not imposing label modifications at this time. Rather, any requirements for product use modifications will occur in the future under the Endangered Species Protection Program.

A. Required Environmental Hazards Statement on the End-Use Product Label

All end-use products formulated from *Bt israelensis* strain EG2215 will be required to have the following statement:

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or public waters unless this product is specifically 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."

V. Summary of Required Data

All hypersensitivity incidents must be reported to the Agency when/if they occur.

As a condition of registration (due within 6 months of registration), the registrant of *Bt israelensis* strain EG2215 is required to conduct a *Daphnia* study with a 10 day exposure period using a maximum hazard dose. This test is required of all registrants of *Bt* products to ensure that their manufacturing processes has been optimized to sufficiently prevent production of significant amounts of heat labile exotoxins. Should this test show significant lethality, a dose response *Daphnia* test must be performed to derive an LC_{50}. Pending the results of this assay, further non-target species testing may be required by the Agency.

As a condition of registration (due within 6 months of registration), the registrant of *Bt israelensis* strain EG2215 is required to conduct an Intraperitoneal injection study. This test is required as part of the product identity data requirements (151A-12C) to demonstrate lack of mammalian toxicity in the Technical Grade of the Active Ingredient (TGAI). The assay should be conducted using mice with three doses (10^6, 10^7, 10^8 CFU per animal) and mortality should be recorded. If the results from this test show significant mortality, additional data may be required by the Agency.

As a condition of registration (due within 6 months of registration), the registrant of *Bt israelensis* strain EG2215 is required to submit the results from a mosquito bioassay to verify the potency (ITU) of the toxin. This test, part of the product characterization, is needed specifically for the toxin and was lacking from the original submission.

As a condition of registration (due within 6 months of registration), the registrant of *Bt israelensis* strain EG2215 is required to complete an eye irritation study (81-4) and a dermal irritation study (81-5). These studies are required to confirm lack of irritation in the final technical product. Until these tests are completed, all products containing *Bt israelensis* strain EG2215 must be labeled with Toxicity Category II statements for eye

VII. Additional Contact Information:

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)
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Environmental Protection Agency
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Washington, D.C. 20460