Bacillus thuringiensis subspecies *kurstaki* strain M-200 (006452) Fact Sheet

1. Description of the Microbial Pesticide

- **Generic Name:** Bacillus thuringiensis subspecies kurstaki strain M-200 Bacillus thuringiensis subspecies kurstaki strain M-200
- **Trade and Other Names:** Able™, CGA-269941
- **OPP Chemical Codes:** 006452
- **Year of Initial Registration:** 1996
- **Pesticide Type:** Insecticide
- **U.S. and Foreign Producers:**

  Thermo Trilogy Corporation
  7500 Grace Drive
  Columbia, MD 21044

2. Use Sites and Application Timing

Bacillus thuringiensis subspecies *kurstaki* strain M-200 is approved for manufacturing-use and for the control of lepidoterous pests of tree fruits, terrestrial small fruits and vegetables, tree nuts, alfalfa, corn, cotton, soybeans, peanuts, herbs and spices, and cranberries. Able™ may be ground or aerially applied and used via the chemigation method of sprinkler irrigation.

3. Science Findings

A. Microbe Description

Two of the toxins have molecular weights of approximately 130 KDa and 60 KDa for the third.

The active ingredient of ™ is identified as *Bacillus thuringiensis* subspecies *kurstaki* strain M-200.

History: *Bacillus thuringiensis* subspecies *kurstaki* strain M-200 was isolated in the United Kingdom.

General Taxonomy: *Bacillus thuringiensis* subspecies *kurstaki* is a gram-positive aerobic rod containing a crystalline protein in the shape of a bipyramidal parasporal crystal; and an elliptical endospore. The flagella antigen serotype was determined to be H3a3b as previously described (de Barjac). This strain produces three insecticidal toxins.

B. Human Health Effects

No unreasonable adverse effects to human health are expected from the use of Able™.

1. Risks Posed by Potential Residential, School or Daycare Exposure

No residential, school or daycare uses currently appear on the label. The use sites are all agricultural for use on growing plants to control lepidopterous pests. Therefore nondietary exposure to these sites, where children are present, are minimal to nonexistent.

2. Effects on the Immune and Endocrine Systems

The active ingredient is a microorganism. No known metabolite that acts as an "endocrine disrupter" is produced by this microorganism. The toxicity/pathogenicity studies in the rodent required for microbial pesticides, as designed, show that after several routes of exposure; the intact immune system is able to process and clear the active ingredient, as expected from non-pathogenic microorganisms. Therefore, no adverse effects to the endocrine or immune systems are known or expected.

3. Potential for the Transfer of the Pesticide 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 *Bt* applied to enter ground water or other drinking water sources, the amounts 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. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of exposure to *Bt* through drinking water. Therefore, the potential of significant transfer to drinking water is minimal to nonexistent.

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

A battery of acute toxicity/pathogenicity studies is considered sufficient by the Agency to perform a risk assessment for microbial pesticides. To date, none of the active microbial pesticidal ingredients registered by the Agency have required subchronic or chronic exposure studies. Also, for food uses of microbial pesticides, the acute toxicity/pathogenicity studies have allowed for the conclusion that an exemption from the requirement of a tolerance is appropriate and adequate to protect human health, including that of infants and children. The results of testing done on Able™ agree with this.

Quality control procedures in place during manufacturing ensure that harmful levels of contaminating microorganisms are prevented and the mammalian toxin beta-exotoxin is not present.

In considering risk to microbial pesticides, it is important to keep the ubiquity of microbes in mind. Most microorganisms are considered to be non-pathogenic for humans, despite the continual exposure to high numbers of a myriad of airborne, waterborne, food- and soil-associated microorganisms as well as human and mammalian commensal microbes ever day.

Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been 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. Several reports under FIFRA Â§ 6(a)2 have been made for non-dietary exposure to certain *Bacillus thuringiensis* products with potential allergic reactions being reported. These reactions were determined by the Agency not to be due to *Bacillus thuringiensis* itself or any of the Cry toxins.

5. Cumulative Exposure From Multiple Routes Including Oral, Dermal & Inhalation

a. Skin would primarily be a route of exposure for mixer/loader applicators. Unbroken skin is a natural barrier to microbial invasion of the human body.

The only way in which skin could be a significant route for exposure would be if the skin were cut, or the microbe was a pathogen with mechanisms for entry through or infection in the skin, or if metabolites were produced that could be dermally absorbed. Bt is not known to be human pathogen nor is it known to produce metabolites that are dermally absorbed. Since the intravenous study demonstrated no adverse effects, even cut skin should not pose a risk to health via entry of Bt into the body.

b. Inhalation would primarily be a route of exposure for mixer/loader applicators. Because the pulmonary study showed no adverse effects, the risks anticipated for this route of exposure are minimal.

c. Oral exposure would result primarily from eating treated produce.

Overall, the combined dermal and inhalation exposure via mixing, loading and applying Able™and oral exposure via eating treated produce or drinking water containing Bt should fall well below the levels tested.

6. Bacillus thuringiensis subspecies kurstaki strain M-200

Toxicity/Pathogenicity

a. Acute Oral Toxicity/Pathogenicity (152A-10)

CGA-269941 Technical was not toxic, pathogenic or infective when an oral dose of approximately 3.65 x 10^8 CFU was given to rats by oral gavage. A distinct clearance pattern was observed by day 7 from all tissues and organs.

CLASSIFICATION: ACCEPTABLE

b. Acute Pulmonary Toxicity/Pathogenicity (152A-12)

MRID No. 435099-12 & 437928-03

CGA-269941 Technical was not toxic, pathogenic or infective when 5 x 10^7 CFU's were administered intratracheally to rats. A distinct pattern of clearance was evident by the end of the study.

CLASSIFICATION: ACCEPTABLE

c. Acute Intravenous Toxicity/Pathogenicity- Rat (152A-13)-

MRID No. 435099-13 & 437928-04 :

CGA-269941 Technical was not toxic, pathogenic or infective when 4 x 10^6 CFU's were administered intravenously to rats. A distinct pattern of clearance was evident by the end of the study.

CLASSIFICATION: ACCEPTABLE

7. Able™ Product Specific Acute Toxicity

a. Acute Oral Toxicity (81-1) - MRID No. 435099-05

The LD_{50} for Able™ was determined to be greater than 5500 mg/kg rat body weight.

CLASSIFICATION: ACCEPTABLE- TOX CATEGORY IV

b. Acute Inhalation Toxicity Study-Rat (81-3) - MRID No. 435099-08:

The LC_{50} of aerosolized Able™ was determined to be greater than 5.63 mg/L when administered to albino rats.

CLASSIFICATION: ACCEPTABLE- TOX CATEGORY IV

c. Acute Dermal Toxicity- Rabbit (81-2) - MRID No. 435099-07:

The acute dermal LD_{50} was greater than 2020 mg/kg when Able™ was applied to the skin of rabbits.

CLASSIFICATION: ACCEPTABLE- TOX CATEGORY III

d. Primary Dermal Irritation - Rabbit (81-5) - MRID No. 435099-09:

CGA-269941 50 WP-A FL-941606 produced a slight irritation (primary irritation index of 0.3/8.0) when a 500 mg dose was administered dermally to rabbits. Dermal irritation was no longer present within 1 hour post-dosing.

CLASSIFICATION: ACCEPTABLE - TOX CATEGORY IV

e. Primary Eye Irritation (152A-14) - MRID No. 435099-15:

Able™ displayed a mild irritation (total Draize score: 5.7 in unwashed eyes and 5.3 in washed eyes) at 24 hours post-dosing, when a single 100 mg (8.33×10^8 CFU) ocular dose was administered. Ocular irritation dissipated by day 4

CLASSIFICATION: ACCEPTABLE- TOX CATEGORY III

8. CGA-269941 Technical (Product Specific Acute Toxicity

a. Acute Oral Toxicity (81-1) - MRID No. 435099-06:

The LD_{50} for CGA-269941 Technical FL-930545 was determined to be greater than 5500 mg/kg rat body weight.

CLASSIFICATION: ACCEPTABLE- TOX CATEGORY IV

b. Acute Dermal Toxicity/Irritation Rabbit (152-11)

MRID No. 435099-11

The primary irritation index was determined to be 3.1; which is equivalent to a score of moderately irritating. The acute dermal LD_{50} was greater than 2020 mg/kg when CGA-269941 Technical FL-930545 was applied dermally to the skin of rabbits.

CLASSIFICATION: ACCEPTABLE - TOX CATEGORY III

c. Primary Eye Irritation - Rabbit (152A-14) - MRID No. 435099-16

CGA-269941 Technical FL-930545 displayed a mild irritation (total Draize score: 12.7 in unwashed eyes) at 1 hour post dosing, when a singular 100 mg (7.7×10^{10} CFU) ocular dose was administered. Ocular irritation dissipated by day 7.

CLASSIFICATION: ACCEPTABLE - TOX CATEGORY III

9. B.T. TOLERANCE EXEMPTION APPLICABILITY

The existing tolerance exemption for *B. thuringiensis* (40 CFR 180.1011) is applicable to strain *B. thuringiensis* subspecies *kurstaki* strain M-200.

C. Ecological Effects

1. General Conclusions

The risk of *Bacillus thuringiensis* subspecies *kurstaki* strain M-200 is minimal to nonexistent to nontarget organisms including endangered species except endangered insect species.

A label limitation to terrestrial use was necessary since 1) an aquatic risk level of concern was triggered for endangered species based on the freshwater aquatic invertebrate study, and 2) only one freshwater fish species was tested and minimal toxicity was observed.

2. Endangered Species Conclusions

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

Prior to the implementation of the Endangered Species Protection Program, the Agency will not impose specific labeling on those pesticides that pose risks to threatened and endangered species and their habitats but will defer imposing specific labeling language until the implementation of The Act. To address this issue in the short term, the following language was be placed in the notices of pesticide registration:

The Agency has concerns about the exposure of threatened and endangered species. Currently, the Agency is developing a program (The Endangered Species Protection Program) to identify all pesticides whose use may cause potential adverse impacts on endangered and threatened species and to implement mitigation measures that will eliminate the adverse impacts. The program will require users to consult county-specific bulletins. These bulletins will provide information about specific use restrictions to protect endangered and threatened species in the county of specific pesticide use. Consultations with Fish and Wildlife Service will be necessary to assess risks to newly listed species or from, proposed new uses.

The Agency plans to publish a description of the Endangered Species Protection Program in the Federal Register and have enforceable county-specific bulletins. Because the Agency is taking this approach for protection of endangered and threatened species, it is not imposing label modifications at this time. Rather, any requirement for product use modifications will occur in the future under the Endangered Species Program."

3. Specific Test Organism Conclusions

Able™ technical powder appears to be practically nontoxic to birds, the sheepshead minnow, grass shrimp, the hymenopterous parasite *Nasonia vitripennis* and the neuropteran predator *Chrysopa carnea*. However, it appears to have some toxicity to freshwater aquatic invertebrates (*Daphnia magna*), bluegill sunfish, the ladybird beetle, *Hippodamia convergens* and honeybees.

Although the data requirement for freshwater aquatic invertebrates (154A-20) has been satisfied, the study was found to be supplemental because toxicity was observed, and a precise LC_{50} was not determined. The data requirements for birds (154A-16), estuarine and marine animals, insect predators and parasites (154A-23) and honeybees (154A-24) were satisfied. The applicant requested a waiver for the required rainbow trout study on the basis that completed bluegill sunfish and sheepshead minnow studies demonstrate substantial margins of safety for the proposed aquatic uses. The waiver request was denied on the basis of significant mortality and statistically significant growth reduction in bluegill sunfish. Since the aquatic use site of watercress was deleted from the product label, a rainbow trout study is not required. However, the addition of aquatic use sites in the future will require the submission of an acceptable rainbow trout study.

1. Birds

154A-16. Avian oral pathogenicity/toxicity test -

MRID No. 435830-02. The study adequately demonstrates that AbleTM technical material when administered by oral gavage at 1.6 g/kg for five days (8 g/kg total) is practically nontoxic to bobwhite quail. The study was classified as satisfactory.

154A-16. Avian oral pathogenicity/toxicity test -

MRID No. 435830-03. The study adequately demonstrates that AbleTM technical material when administered by oral gavage at 1.6 g/kg for five days (8 g/kg total) is practically nontoxic to mallard ducklings. The study was classified as satisfactory.

2. Aquatic Invertebrate

154A-20. Freshwater aquatic invertebrate toxicity and pathogenicity testing - MRID No. 435830-05. The study does not satisfy 154A-20 requirements because accurate LC_{50} and 95% confidence limits [Sections (b)(7)(i) and (c)(1) 154A-20 Subdivision M] were not determined for Able^{TM} technical powder, which in preliminary testing had killed 100% of daphnids exposed to the maximum hazard concentration of 1,000 times the EEC of the material. The study did establish a lowest observable effect concentration (NOEC) of 1.1 X 10^9 cfu/L, a reproductive NOEL of 5.6 X 10^8 cfu/L, and that the LC_{50} is > 1.1 x 10^9 cfu/L. Although the study was classified as supplemental, nontarget organism risk quotients could be calculated. Using an estimated environmental concentration of 9.6 x 10^7 cfu/L, the risk quotients were calculated to be: acute risk -> 0.087 ; chronic risk -> 0.17. These figures demonstrate levels of concern being reached for acute effects on endangered species. However, since the EEC assumes direct application to water, endangered species concerns are not triggered for terrestrial uses.

154A-21. Estuarine and marine animal toxicity and pathogenicity testing - MRID No. 435830-06. The study adequately demonstrates that Able^{TM} technical powder is practically nontoxic to sheepshead minnow over the 31 day test period with an aqueous LC_{50} > 4.9 X 10^{10} cfu/l of dilution water and an oral LC_{50} > 3.7 X 10^7 cfu/g of food. The aqueous and oral NOELs are > 4.9 X 10^{10} cfu/L of dilution water and 3.7 X 10^7 cfu/g of food, respectively. The study was classified as satisfactory.

154A-21. Estuarine and marine animal toxicity and pathogenicity testing - MRID No. 435830-07. The study adequately demonstrates that

Able™ technical powder is practically nontoxic to grass shrimp when fed food containing 3.6 X 10^7 cfu/g over the 31 day test period. The oral NOEL is > 3.6 X 10^7 cfu/g of food. The study was classified as satisfactory.

3. Fish

154A-19. Freshwater fish toxicity and pathogenicity testing

MRID Nos. 435830-04 & 437900-01. The study demonstrates that technical Able™ material, at the doses tested (4.7 X 10^{10} cfu/l of water and in food containing 3.9 X 10^7 cfu/g), causes statistically significant mortality and statistically highly significant growth reduction with bluegill sunfish. However, with an aqueous LC_{50} > 4.7 X 10^{10} cfu/l of dilution water and an oral LC_{50} > 3.9 X 10^7 cfu/g of food, it is not expected that the use of Able™ according to it's label directions for use would pose a hazard to bluegill sunfish. The study was classified as satisfactory.

4. Honey Bees and Nontarget Insects

154A-24. Honey bee toxicity/pathogenicity test -

MRID No. 435681-01. The study demonstrates that Able™ technical powder is slightly toxic to adult honeybees with a 10-day *per os* LC_{50} of 633 ppm when calculated on the amount the bees were exposed to, and 118 g/bee when calculated on the total quantity actually consumed per bee. However, it is not expected that the use of Able™ according to its label would pose any hazard to honeybees. The no effect concentration (NOEC) and lowest observed effect concentration (LOEC) were 156 and 625 ppm, respectively. According to the honeybee toxicity rating of Atkins, 1976, CGA-269941 is practically nontoxic to adult honeybees. The study was classified as satisfactory.

154A-23. Nontarget insect testing, predaceous neuropteron -

MRID No. 435830-10. The study adequately demonstrates that Able™ technical powder is practically nontoxic to green lacewing larvae per os at a dosage of 3,000 ppm of food when fed for 8 days. The NOEL is 3,000 ppm. The study was classified as satisfactory.

154A-23. Nontarget insect testing, predaceous coleopteran

MRID No. 435830-09. The study demonstrates that CGA-269941 technical powder is very slightly toxic to adult *H. convergens* (Coleoptera, Coccinellidae) per os at a dosage of 3,000 ppm of food when fed for 21 days. However, it is not expected that the use of Able™ according to its label would pose any hazard to predaceous coleopterans. The NOEL is 1,500 ppm. The study was classified as satisfactory.

154A-23. Nontarget insect testing, parasitic hymenopteran -

MRID No. 435830-10. The study adequately demonstrates that Able™ technical powder is practically nontoxic to adult *N. vitripennis* per os at a dosage of 3,000 ppm of food when fed for 15 days. The NOEL is 3,000 ppm. The study was classified as satisfactory.

5. Nontarget Plants

Since the Agency is not aware of any evidence that *Bacillus thuringiensis* causes any adverse effects to plants, the risk to nontarget plants is minimal.

6. Mammals

Based on the mammalian studies reviewed in the health effects assessment, the risk to mammalian wildlife is minimal to nonexistent.

4. Summary of Data Gaps

Data outlined in the *Bacillus thuringiensis* RED, including a new description of the manufacturing process and the 10-day *Daphnia magna* study, are required and currently outstanding.

5. Contact Person at EPA

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460