US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDE REGULATORY ACTION DOCUMENT
HARPIN PROTEIN
(PC Code 006477)

March 31 2002

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U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Updated 3/31/02

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I. EXECUTIVE SUMMARY

A. IDENTITY/MODE OF ACTION

Harpin protein initiates a complex set of metabolic responses in the treated plant, causing natural gene expression and eliciting a plant's natural defense and growth systems. It is isolated from *Erwinia amylovora*, the bacterial pathogen that causes the disease fire blight in apples and pears. Classified as a biochemical pesticide, it is a broad-spectrum fungicide alternative with efficacy against a wide variety of fungal, bacterial, and viral diseases, some of which have no other means of control. The product also aids in the suppression of certain insect, mite, and nematode pests and enhances plant growth. Harpin is commercially produced in *Escherichia coli* by transfer of a DNA fragment encoding harpin protein from *E. amylovora* to the cell production strain, *E. coli* K-12. The harpin producing strain is considered a debilitated strain of *E. coli*, which cannot grow in the human digestive tract, or survive in the environment. *E. coli* K-12 cells are killed and lysed at the end of the fermentation process. Harpin protein and other cell constituents are then extracted for formulation into an end-use product, MESSENGER®.

Harpin exhibits no direct inhibitory or toxic effect on plant pathogens, and thus cannot exert the selection pressure that would promote the development of resistance in pest populations.

B. USE/APPLICATION

Harpin protein is proposed for use on all food commodities in agricultural fields and greenhouses; on turf, trees, and ornamentals. It is formulated as a wettable granule for application with conventional ground or aerial spray equipment, as well as sprinkler, drip or chemigation systems. Use rates are extremely low, generally 2 to 11.5 grams of active ingredient per acre. Harpin must not be diluted/applied with chlorinated water, due to denaturation of the protein.

C. RISK ASSESSMENT

Health Effects

Harpin protein is classified as a Toxicity Category IV product via the oral, dermal and inhalation route, and a Toxicity Category IV eye and skin irritant. For the previous six years, researchers and workers have worked with harpin in its production and application, and there has been no indication of any toxicity or hypersensitivity associated with this protein. Because of the lack of demonstrated adverse health effects, low rates of application, and rapid degradation in the field, no residues are expected on treated crops and attendant dietary risks are expected to be minimal to non-existent. Because of the lack of demonstrable toxicity, no adverse effects are expected to applicators, handlers and other workers. Finally, there is reasonable certainty that no harm to adults, infants or children will result from aggregate exposure to harpin residues.

Ecological Effects

Harpin has no demonstrated adverse effects in bird, fish, *Daphnia*, and algae. In addition, it has no effect on seedling emergence of 10 agronomically important plants, and has been judged to be practically nontoxic to honeybees. Evidence from these studies suggest that the amounts of harpin required to elicit acute toxicities in nontarget organism populations would not likely be achieved by exposures to harpin applied at label rates.

D. DATA GAPS/LABELING RESTRICTIONS

All data requirements for registration under Section 3(c)(5) have been satisfied and found to be acceptable. On April 19, 2000, this biochemical pesticide was granted a conditional registration under the terms of Section 3(c)(7)(C). The registrant was required to complete and submit within a period of 12 months after the issuance date: (1) a *Daphnia* life cycle study to further assess potential impacts on freshwater invertebrates; (2) a five- batch analysis to verify the lack of detection of the Harpin-producing strain of *E. coli* in the end-use product, which includes refinements in the quantitation of potential human pathogens as well as sampling for the presence of any other bacteria, including *E. coli* which have lost the plasmid encoding for Harpin; and (3) studies designed to detect residual plasmid in the end-use product.

All studies required under the terms of the conditional registration were received by the Agency on April 17, 2001. Subsequent review of these studies has found them to be acceptable and full registration under Section 3(c)(5) was granted on April 11, 2002.

Because Harpin protein is classified as a Toxicity Category IV compound for all routes of exposure, no human health precautionary statements are required.

E. PUBLIC INTEREST FINDING

Because of its low toxicity and lack of residues, harpin protein is expected to be an important broad-spectrum alternative to conventional fungicides. As an example, Harpin has been used effectively in tomato Integrated Pest Management (IPM) programs, decreasing usage of conventional fungicides and insecticides by an average of 70%, while controlling diseases as well or better than conventional fungicides. In addition, it has been shown to be effective for controlling certain bacterial and viral pathogens, which currently have no other alternative. Because Harpin's mode of action does not involve direct interaction with the pathogen, and thus is not likely to promote the development of resistance in the pest, Harpin protein is expected to be an important tool in resistance management programs for conventional pesticides.

II. OVERVIEW

A. ACTIVE INGREDIENT OVERVIEW

A.I. Name: Harpin Protein

Product Name: Messenger®

P.C. Code: 006477

Basic Manufacturer: EDEN Bioscience Corporation
11816 North Creek Parkway N.
Bothell, Washington 98011-8205

B. USE PROFILE

Type of Pesticide: Biochemical derived from killed genetically engineered *E.coli* K12

Use Sites: All food commodities in agricultural fields and greenhouses; turf, trees, and ornamentals.
Target Pests: Broad range of fungal, bacterial and viral disease organisms, including bacterial leaf spot (*Xanthomonas campestris)*, bacterial speck (*Pseudomonas syringae)*, bacterial wilt (*Pseudomonas solanacearum*), Fusarium wilt, Phytophthora root rot, stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), apple scab (*Venturia inaequalis*), fire blight (*Erwinia amylovora*), Botrytis bunch rot, black rot *(Guignardia bidwellii*), black leaf spot (*Diplocarpon rosae*), cucumber mosaic virus, root-knot nematodes (*Meloidogyne* spp.), tobacco cyst nematode (*Globodera solanacearum*), and tobacco mosaic virus (TMV). Provides significant plant growth enhancement and suppression of some insects. Growth enhancements may include improved germination, increased overall plant vigor, accelerated flowering and fruit set, advanced maturity, and increased yield and quality of the final harvest.

Formulation Types: Messenger is an end-use product formulation containing 3% Harpin protein, formulated as a wettable granule.

Method and Rates of Application: Conventional ground or aerial foliar or pre-plant spray; seed treatment; application via conventional sprinkler, drip, or chemigation systems, and greenhouse drench application. Use rates are generally 2-11.5 grams of active ingredient per acre at 14-day intervals.

Type of Treatment: Foliar spray; seed treatment; irrigation/chemigation; greenhouse soil drench.

Equipment: Conventional ground or aerial spray equipment; sprinkler, drip irrigation, chemigation systems; traveling boom.

Timing: Greenhouse drench application 3 weeks after seeding and a second 5-7 days before transplanting. Field foliar applications are recommended at planting and 14-day

intervals through harvest. For newly-seeded crop, sprays begin at the appearance of the first true leaf.

Use Practice Limitations: Not to be diluted/applied with chlorinated water, due to oxidative deactivation of Harpin protein.

C. DATA REQUIREMENTS

All data requirements have been satisfied for full registration of this biochemical pesticide under Section 3(c)(5). The Agency reviewed the data required for the proposed uses of this pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as amended by the Food Quality Protection Act (1996). For Harpin protein, the product identity and product analysis information, as well as data submitted to assess acute mammalian toxicology and ecological effects are sufficient to support the proposed use patterns during the period of conditional registration. Based on submitted information, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of this biochemical pesticide as labeled. A Section 3(c)(7)(C) conditional registration of this new active ingredient was granted to allow the registrant sufficient time to generate additional data to verify the lack of pathogenic effects on *Daphnia magna*, as an indicator species of non-target organisms. In addition, the Agency required a five-batch analysis to verify lack of detection of the harpin-producing strain of *E. coli* in the end-use product, which includes refinements in the quantitation of potential human pathogens; and studies designed to detect residual plasmid in the end-use product, as well as the presence of any other bacteria, including *E. coli* which have lost the plasmid. These data required under the terms of the conditional registration have been submitted to the Agency, reviewed, and found acceptable to support full registration under Section 3(c)(5).

D. REGULATORY HISTORY

Experimental Use Permit and Temporary Tolerance Exemption

A 2-year Experimental Use Permit (69834-EUP-1) was issued in October, 1998 and an exemption from the requirement of a temporary tolerance was granted for the duration of the EUP (40 CFR 180.1204). The approved experimental program encompassed a broad range of use sites, including tomatoes, peppers, cotton, cucurbits, rice, strawberries, tobacco, small grains, peanuts, alfalfa, potatoes, grapes, apples, citrus, soybeans, blueberry, cranberry, raspberry, corn, sugar cane, conifer seedlings, turf and ornamentals. The maximum quantity of active ingredient approved was 548.58 pounds, on a total of 4997 acres in 31 states.

Section 3 Registration

Subsequent to the EUP, the registrant filed Pesticide Petition 9F6027 for a tolerance exemption on all food commodities. A Notice of Filing was published in the Federal Register on September 9, 1999 (64 FR 49010). In addition, a Notice of Receipt of an application for a new active ingredient was published on November 26, 1999 (64 FR 66474). No comments were

received in response to either notice.

A conditional registration was issued on April 19, 2000, and the final rule for an Exemption from the Requirement of a Tolerance for Harpin protein was published in The Federal Register on May 3, 2000 (65 FR 25660).

E. FOOD CLEARANCES/TOLERANCES

The Agency evaluated data under Section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA) as amended by the Food Quality Protection Act (FQPA) of 1996. Safety factors were considered for human health effects, as well as aggregate and cumulative exposures. Dietary exposure from the potential of secondary transfer of residues to drinking water during applications of the pesticide was also considered. The data submitted are sufficient to support the exemption from the requirement of a tolerance in or on all food/feed commodities.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

Product Identity:

The Agency has classified Messenger®, containing the active ingredient harpin, as a biochemical pesticide because it is derived from a naturally occurring microorganism, does not contain any persistent microbes, has a non-toxic mode of action, and does not alter the DNA of treated plants. Harpin is produced in *Escherichia coli* K-12 by transfer of a DNA fragment encoding one of the harpin proteins from *Erwinia amylovora* to the cell production strain. The harpin producing strain is considered a debilitated strain of *E. coli* which has no mammalian gut colonization potential and is reliant upon laboratory conditions for survival. Cells are killed and lysed to release harpin protein and other cell constituents for formulation into an end-use product.

Product chemistry data used in support of the registration of Messenger® are summarized in Table 1.

Table I. Physical and Chemical Properties for Messenger®

Guideline Number	Study	Result	MRID Number
OPPTS 880.1100	Messenger® Product Analysis;	Acceptable. Proper quality control measures are in place to detect contaminants	445424-01 448702-01

OPPTS 880.1100	Detection of Endotoxin Formation	Acceptable. Contribution of endotoxin from the inerts is minuscule relative to the contribution from a typical water supply. Endotoxin at these amounts is not considered dangerous to human health or to non-target organisms.	448702-14
OPPTS 880.1200	Summary of Production Process Used to Produce Messenger ; Supplemental Information; Quality Control Guidelines for Detection of Human Pathogens in Messenger	Acceptable. Applicable rejection limits for microbial contaminants are part of the manufacturing process.	446599-01 446546-01 450817-01
OPPTS 885.1300	Analysis of Potential Resistance Genes from Messenger	Supplementary. No transformants were detected in assays to detect residual DNA in the end-use product. Recommendation: The registrant needs to apply a PCR-based amplification reaction to give an estimate of the residual plasmid and potential for intact antibiotic resistance genes remaining in Messenger.	448702-16
OPPTS 885.1300	Harpin Messenger Product Analysis Data and Information; Formation of Unintentional Ingredients.	Acceptable. Harpin is produced in a debilitated lab strain of *E. coli.* No unintentional ingredients of a harmful nature were detected in manufacturing.	445424-01
OPPTS 880.6302 880.6303	Color, pH, and Physical State of Messenger.	Acceptable. Messenger is a fine granule of pH 7.86 (1 % solution) at 22 °C; no foreign matter was noted.	448702-02
OPPTS 880.7300	Bulk Density of Messenger™.	Acceptable. Mean bulk density was 0.452 g / mL at room temperature	448702-03

OPPTS 880.1400	Characterization of Test Substances. Characterization of Test Substances Used in Toxicology Studies	Acceptable. Acute oral toxicity studies performed with 0.3% (TGAI) and 19 % harpin (concentrate);oral, dermal and eye irritation, and inhalation studies done with 3% harpin (EP).	445424-02 447449-01 447449-02 447449-03 447449-04 447449-05
OPPTS 880.1300 880.1400	Analysis of Residual Antimicrobial Activity in Messenger.	Acceptable. Antibiotic sensitive bacterial strains demonstrated the lack of significant levels of residual antibiotic from the growth medium in the product.	448702-15
OPPTS 885.1400	Survival of Messenger Production Cell Line in Reconstituted 3 % Messenger.	Supplementary, upgradable: Reconstituted Messenger solution contained no detectable *E. coli* K-12 cells, although other bacteria were detected. Recommendation: The registrant should repeat this procedure and include LB and EMB plates without antibiotics for comparison.	449537-01
OPPTS 885.1400	Quantification of Agglomerated 3% a.i. Messenger lots PR98151, PR99021, PR9902, PR99024, PR99026, and PR99027.	Acceptable. The range of harpin concentration was 28.51 to 32.00 mg/g of product.	445424-02 448702-06
OPPTS 880.1600	Certification of Limits	Acceptable. CSF limits are adequate.	448702-01
OPPTS 880.1700	Enforcement Analytical Method	Acceptable	450749-01
OPPTS 880.7000 880.7050 880.7300	Determination of the Water Content in Five Batches of Messenger Biopesticide; Determination of the Ultraviolet-Visible Absorption Spectrum of Harpin.	Acceptable. Messenger has a maximal absorbance at 204 nm (pH 7) and a mean water content of 2.60 %.	448702-04 448702-05

Conditional Registration Data Requirements

The following quality control studies were submitted under the terms of the Section 3(c)(7)(C) conditional registration, reviewed by the Agency, and found to be acceptable.

(1) A five-batch analysis to verify the lack of detection of the Harpin-producing strain of *E. coli* in the end-use product, which includes refinements in the quantitation of potential human pathogens as well as sampling for the presence of any other bacteria, including the production strain of *E. coli*, which have lost the plasmid encoded for Harpin.

MRID# 45380402. Bacterial Analysis of Five Messenger Lots.
The five-batch analysis was performed in order to determine the level of bacterial contamination, including human pathogens, as well as live production cells remaining in the end-use product. Levels of all these potential contaminants was low and within the limits expected for this type of manufacturing scheme and comparable to other biological pesticides.

(2) A PCR-based amplification study designed to detect residual plasmid in the end-use product.

MRID# 45380403. Detection and Quantification of Plasmid DNA in Messenger.
The quantity of intact and fragmented production plasmid remaining in the end-use product was determined to be minuscule and not expected to provide for gene transfer to other soil microbes.

B. HUMAN HEALTH RISK ASSESSMENT

The acute oral, dermal and inhalation toxicity studies were conducted according to Agency guidelines and demonstrated no significant adverse effects from dosing with harpin protein. Following from this, there is a reasonable certainty of no harm from exposure to harpin or Messenger®. All anticipated dietary and other exposures for which there are reliable information are included in this assessment.

Although *Escherichia coli* has been implicated as a human pathogen following consumption of contaminated water or food, this trait is highly strain specific and does not include the cell production strain used for harpin or other K-12 derivatives. The cell production strain does not produce any known virulence factors and is deficient in the ability to attach to the mucosal lining of the mammalian gut. No known toxins are secreted from the cell production strain that would suggest it has the potential for pathogenicity or toxicity to humans or other animals. Steps are taken in the production and packaging process to eliminate viable cells such that *E. coli* K-12 is non-detectable in Messenger® and therefore exposure to viable cells during application of Messenger® is highly unlikely. Additionally, attempts to detect viable cells on treated plants indicates that the cell production strain does not survive the normal mixing and application process for this biopesticide.

For the previous six years, workers at EDEN and University labs have worked with harpin in production situations, and in greenhouse and field applications. There has been no indication of

any toxicity or hypersensitivity associated with this protein. For the past four years, Messenger® has been produced at EDEN without any evidence of a hypersensitive reaction in the twenty-plus people who have worked with it. In total, at least 150 people have been involved with Messenger® in field trials in the United States, Mexico and the Peoples Republic of China, as cooperators with EDEN, and no incidents have been reported.

Production workers at EDEN have been exposed to harpin at much higher levels than would be present in field applications of Messenger® and, to date, no evidence of hypersensitivity has been observed. Monitoring of production workers is ongoing at EDEN and any adverse reactions will be reported through the mandated FIFRA 6(a)2 reporting guidelines. Workers at EDEN have been subject to the same precautionary use of dust masks as is prescribed for mixers in the field. The lack of incidents suggests that harpin is not likely to be an allergen.

Based upon the lack of demonstrable toxicity and the non-pathogenic nature of K-12 derivatives, it is not anticipated that any human health consequence will result from application of Messenger®. Additionally, mixers are directed to wear personal protective equipment including a dust mask (N-95, P-95, R-95) to preclude inhalation of aerosolized carrier, protein, or residual fermentation biomass.

1. Human Toxicity Assessment

a. Acute Toxicity

All the required mammalian toxicology data requirements have been submitted and adequately satisfy data requirements to support registration. Three acute oral toxicity studies were conducted, two at the limit value of 2g/kg body weight and one at the limit value of 5 g/kg, placing this product in Toxicity Category IV. Inhalation toxicity, and dermal toxicity studies also resulted in Toxicity Category IV assessments. Eye and skin irritation studies indicate that Messenger® is not considered a significant ocular or dermal irritant of rabbits, although initial studies indicated that Messenger® is a mild irritant (toxicity category III). Dermal and eye irritation studies were repeated with an improved formulation of Messenger®, thereby reducing irritation, and resulting in a classification of Toxicity Category IV for both routes of exposure.

Table 2. Toxicity Data Requirements

Guideline Number	Study	Result	
OPPTS 870.1100	An Acute Oral Toxicity of Study of Harpin Protein Technical in Rats.	Acceptable. (0.3 % harpin) LD_{50} is > 2 g/kg body weight. Toxicity category III. Acceptable. (19% harpin) LD_{50} is > 2 g/kg body weight. Toxicity category III.	445424-03 445424-04 447449-01 447449-02
OPPTS 870.1100	An Acute Oral Toxicity / Limit Testing of Messenger Following Acute Oral Challenge.	Acceptable. (3% harpin) The acute oral toxicity of Messenger™ is > 5 g/kg body weight. Toxicity category IV.	448702-07
OPPTS 870.1200	Acute Dermal Toxicity	Acceptable. The acute median lethal dose or LD_{50} for Messenger in adult rabbits is > 6 g/kg body weight. Messenger™ is considered a mild dermal irritant. Toxicity category IV.	448702-08
OPPTS 870.2500	Acute Dermal Irritation	Acceptable. Slight erythema, desquamation, fissures and slight edema were noted, but cleared by 72h. Toxicity category III.	445424-07 447449-05
OPPTS 870.2500	Acute Dermal Irritation	Acceptable. No mortality was noted during this study and no signs of skin corrosiveness or dermal irritation were noted. Erythema, edema and eschar scores were all 0 at all time points for all animals. Messenger™ is not considered a dermal irritant of rabbits. Toxicity category IV.	448702-11
OPPTS 870.1300	Acute Nose- only inhalation Study	Acceptable. No adverse effects noted: LC_{50} is > 2 mg/L. Toxicity category IV.	445424-05 447449-03

OPPTS 870.1300	Acute Nose- only inhalation Study	Acceptable. Gross necropsy indicated that four of the animals had no gross lesions or other notable findings, but six had mottled red lungs and two also had multiple red foci. These findings were considered as being treatment related. LC_{50} is > 2.16 mg/L. Toxicity category IV.	448702-09
OPPTS 870.2400	Primary Eye Irritation Study	Acceptable. Conjunctival redness noted in 5/6 rabbits clearing by 72h. Toxicity category III.	445424-06 447449-04
OPPTS 870.2400	Primary Eye Irritation Study	Acceptable. No corneal opacity, iridal lesions, conjunctival chemosis or erythema were noted. Messenger is not considered an ocular irritant of rabbits. Toxicity category IV.	448702-10
OPPTS 885.3400 (152-16)	Hypersensitivity Incidents - Human Exposure	Acceptable. The current observations suggest that harpin is not likely to be allergenic.	445424-08 448702-12
OPPTS 870.2600	Hypersensitivity Study	Waived because of worker exposure history.	
OPPTS 870.5000 to 870.5915	Genotoxicity Testing	Waived	
OPPTS 870.7800	Immune Response	Waived	
OPPTS 870.3100	Subchronic Feeding Study	Waived	
OPPTS 870.3700	Teratogenicity Study	Waived	

OPPTS 885.3400	Human Health and Environmental Risk Assessment of Messenger™ Production Cell Line.	Acceptable. With the use of a low toxicity protein in small amounts to incite plant defense reactions, no significant human health concerns are warranted.	448702-13

b. Subchronic Toxicity and Chronic Toxicity

Hypersensitivity testing was waived and replaced with the requirement for reporting hypersensitivity incidents in workers, handlers, and other individuals repeatedly exposed to harpin protein during research, testing, and field use of the product. Production workers at EDEN have been exposed to harpin at levels approximately 600 times greater than those which would occur during actual field use under label conditions and to date, no evidence of hypersensitivity has been observed. In addition, over a period of ten years, more than 150 people have been involved in the research, development and field testing of harpin protein internationally, and no hypersensitive effects have been noted.

Immunotoxicity, teratogenicity, genotoxicity and subchronic feeding studies have been waived for the following reasons: 1) The proteinaceous nature of harpin, in combination with its lack of demonstrable toxicity in acute studies, contributes a level of safety since proteins which are known to be chronically toxic can be demonstrated to be acutely toxic at high dose levels (Sjoblad, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," *Regulatory Toxicology and Pharmacology*, 15, 3-9). Therefore, because no significant adverse effects were observed in acute studies, even at the limit doses, harpin is not considered to be an acutely toxic protein. 2) Repeated (subchronic or chronic) dietary exposure is highly unlikely because residues are undetectable even immediately after application due to the extremely low application rates (approximately 2-11.5 grams of protein per acre) and because harpin protein is rapidly degraded by microbial and oxidative agents (e.g., chlorinated water) as well as by sunlight, as has been shown by environmental fate and residue data. 3) Because harpin protein is inactivated by chlorine, if any residues were present, they would be rapidly degraded by washing in municipally treated water, which typically contains chlorine. 4) In addition to the above arguments, genotoxicity studies were not required since these studies are typically done with bacterial or mammalian cell cultures. The harpin product would directly interfere with the growth of these cultures either by providing nutrients to supplement the growth of the bacterial mutants or enhancing proliferation of the mammalian cell cultures. 5) Survival, replication, toxicity and persistence of the harpin producing strain of *E. coli* or its products was not observed in any of the toxicity studies, including inhalation, oral, dermal and eye exposures. The Office of Pollution Prevention and Toxics (OPPT) completed a risk assessment for *E. coli* K-12 in 1994, in which it is stated that because of this organism's wide use as a model in research in microbial genetics and physiology, as well as its use in industrial applications, *E. coli* K-12 is one of the most extensively studied microorganisms, and has a considerable history of safe use. Moreover, the *E. coli* K-12 cell production strain does not survive the normal mixing and application process for this biopesticide.

For all the above reasons, the Agency believes that no further subchronic or chronic testing is necessary to characterize the toxicological properties of harpin protein or its end-use product Messenger®.

c. Effects on the Immune and Endocrine Systems

The Agency has no data to indicate that the active ingredient harpin or the end-use product Messenger® have any effect on the immune or endocrine systems. Due to the low or lack of toxicity observed in studies following oral, pulmonary, dermal and ocular exposure, it is unlikely that harpin has any endocrine effects on man or other animals. Hence, the Agency is not requiring any studies on the endocrine system at this time.

2. Dose Response Assessment

No toxicological endpoints are identified.

3. Dietary Exposure and Risk Characterization

The use of Messenger® is not expected to result in any new dietary exposure to this protein. Harpin and related harpin proteins are common constituents of plant pathogenic bacteria which are often found on fruits and vegetables. The quantities of Messenger® applied to crops is very small and residues are virtually undetectable soon after treatment due to the instability of harpin protein in the environment. Hence, the increase of harpin-like proteins expected from the use of Messenger® is minuscule relative to the natural occurrence of these proteins.

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

a. Occupational Exposure and Risk Characterization

During the preparation and application of Messenger® the primary routes of exposure to the mixers and applicators would be through dermal and pulmonary routes. Harpin is not considered to be a dermal irritant and the natural barrier of the skin would preclude significant absorption of this protein. Any residual cells of the production strain of *E. coli* will similarly be excluded by the dermal barrier as this strain is not known to be invasive, pathogenic or capable of producing toxins that might be dermally absorbed. Pulmonary toxicity tests, while placing this product in Toxicity Category IV, indicate that exposure to high levels of the end-use product may result in treatment-related lung lesions. Therefore, use of a dust mask is indicated for mixers of this product. Use of such personal protective equipment will preclude exposure from the inhalation route. Based upon the results of acute toxicity tests and the small quantities of product applied, risks from dermal or pulmonary exposures are considered to be minimal.

b. Residential, School and Day Care Exposure and Risk Characterization

The approved use of Messenger® for greenhouses, field crops, and commercial application to turf, trees and ornamentals should not result in significant increased exposure to residences, schools or day care institutions. Due to the intended sites of application, the methods of application and the small quantities applied, risks to humans, including children, are considered to be negligible. Based upon the results of acute toxicity tests and the small quantities of product applied, risks from oral, dermal or pulmonary exposures are considered to be minimal.

5. Drinking Water Exposure and Risk Characterization

Harpin protein and closely related molecules are normal constituents of many bacteria present in the environment and can be expected to be found in water and other places in the environment. The cell production strain of *E. coli* is an attenuated laboratory strain not known to be pathogenic to animals or plants, or a producer of toxins. The potential for exposure to this bacterium or its products is negligible because the bacteria are killed and removed during the manufacturing process. Moreover, even if extremely small numbers of this bacterium were present in the end use product, *e.g.,* below the level of detection, studies demonstrate that (1) the *E. coli* K-12 cell production strain does not survive the normal mixing and application process for this biopesticide; and (2) the bacterium is unable to survive in the environment. Because harpin protein is applied at extremely low use rates and rapidly degrades in the environment, residues are unlikely to occur in ground or surface water. In addition, the strain used for harpin production and the protein itself are both susceptible to degradation by chlorine, and as such would not be found in typical municipal drinking water. The inert ingredients included in the end-use product are also non-toxic and may be found in some food products.

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

No reports of allergic reactions have been reported for the *E. coli* cell production strain or harpin proteins and reports of production workers and researchers exposed to these entities indicate no reports of hypersensitivity.

Based upon the toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure of the United States population, including infants and children, to residues of harpin protein and of its end-use product, Messenger®. This includes dietary and all other exposures for which there is reliable information. The Agency has reached this conclusion based on the results of the toxicity studies and available literature which indicate that *E. coli* K-12 derivatives and harpin are practically non-toxic to mammals and under reasonably foreseeable circumstances they do not pose a risk.

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

The application of Messenger® to field and greenhouse crops occurs at extremely low rates and the residues on crops soon after treatment are minuscule to non-detectable. The active ingredient, harpin, is unstable in sunlight, heat and chlorination and is not expected to persist in the environment. Due to this lability, the lack of significant toxicological concerns, as demonstrated by the Agency's evaluation of mammalian toxicological studies, and the low use rates, the potential risks to humans are considered negligible.

8. Cumulative Effects

Messenger® and its active ingredient, harpin, are practically non-toxic to mammals. No mechanism of toxicity in mammals to this protein or the cell production strain of *E. coli* have been identified, hence, no cumulative effect is anticipated.

C. ENVIRONMENTAL RISK ASSESSMENT

1. Ecological Toxicity

a. Toxicity/Pathogenicity to Nontarget Organisms

Data were submitted on avian oral, avian dietary, rainbow trout, daphnia, and algal toxicity, seedling emergence of 10 agronomically important plants and honeybee contact acute toxicities for the active ingredient, harpin. Evidence from these studies suggests that the amounts of harpin required to elicit acute toxicities in nontarget organism populations would not likely be achieved by intended exposures to labeled applications.

Harpin is produced by fermentation of a weakened strain (K-12) of the bacterium, *Escherichia coli.* BPPD believes that risks to nontarget organism populations will be minimal to nonexistent as a result of intended applications of *E. coli* K-12, encoded to produce harpin which may have survived the manufacturing process. Several lines of presumptive evidence led to this present assessment: **(1)** The K-12 strain of *E. coli* is considered as a nutritionally deficient bacterium, unlikely to have competitive capabilities required for survival in the environment.
(2) The OPPTS risk assessment of 1994 concluded that the use of *E. coli* K-12 under contained conditions in fermentation facilities presented low risk, based upon its history of safe use, its classification as a Class 1 Agent under NIH Guidelines (National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules, 1986); as well as other information and assessment of the open literature. As a Class 1 Agent under Section III D-4, NIH exempts transfers of genetic material between species that exchange DNA by known physiological processes with the genus *Escherichia* (including exchanges with related genera of *Shigella, Salmonella, Enterobacter, Citrobacter, Klebsiella, Erwinia*, and others). **(3)** A census of the open literature revealed negative results with respect to infectivity and pathogenicity of *E. coli* K-12 strains to mice, pigs, chickens, and calves. **(4)** The manufacturer submitted additional scientific evidence supporting minimal adverse effects based on supplementary "ecological" studies of the harpin-producing strain of *E. coli,* which is included in the section regarding

"Environmental Fate and Ground Water Data".

Table 3 . Non-Target Toxicity (as of February, 2000)- Tier I Guideline Requirements for Harpin

OPPTS Guideline No.	STUDY	RESULTS	MRID Nos.
850.2100	Avian Acute Oral Toxicity	(1) LD_{50} >4,000 mg messenger/kg body weight; NOEC =4,000 mg messenger/kg body weight ; (2) LD_{50} > 2,250 mg/kg harpin/kg body weight	(1) 448702-18; (2) 445424-09
850.2200	Avian Dietary Toxicity	(1) LC_{50} >100,000 mg messenger/kg body weight; (2) LC_{50} > 5,620 mg harpin/kg body weight	(1) 448702-19; (2) 445424-10
850.1075	Cold Water Fish Acute Toxicity	LC_{50} >3,270 mg messenger/L; NOEC = 378 mg Messenger/L	448702-20
870.2400	Aquatic Invertebrate Acute Toxicity	(1) EC_{50} = 1173 mg Messenger/L; NOEC = 325 mg Messenger/L; (2) EC_{50} > 120 mg harpin/L (nominal)	(1) 448702-21; (2) No MRID
850.3020	Acute Contact Toxicity with the Honeybee	LD_{50} >39 µg harpin/bee; NOEC = 39 µg harpin/bee	449459-01
850.4100; 850.4225	14-day Seedling Emergence	NOEC = ~1430 L/ha (~143 g Harpin/ha)	448702-22
850.4025	Non-Target Plant Studies	Waived	
850.2600	Non-Target Insect Studies	Waived	
850.5400	Freshwater Algal Acute Toxicity	EC_{50} = 182 mg Messenger/L; NOEC = 120 mg Messenger/L	448702-23

2. Conditional Registration Data Requirements

The following study was submitted under the terms of the Section 3(c)(7)(C) conditional registration, reviewed by the Agency and found to be acceptable.

MRID# 45380401. A 21-Day Life Cycle Toxicity and Pathogenicity Study with *Daphnia magna*.

The NOEC was determined to be greater than 1.58×10^5 cfu/mL, the highest concentration tested. This study fulfills the OPPTS Guideline 885.4240 testing requirement for freshwater invertebrate hazard assessment.

3. Environmental Fate and Ground Water Data.

Several studies addressing Agency concerns regarding residual viable organisms in the end-use product were submitted as supplemental to the required Tier I test requirements (Table 3a). These studies provided sufficient scientific evidence that intended exposures of harpin to nontarget organisms are likely to be minimal. Furthermore, the protein is produced naturally by a number of Gram-negative bacteria, and it was shown that it degrades rapidly from plant surfaces (less than 10 days).

The strain of *E. coli* used to produce harpin, is a nutritionally-deficient, environmentally debilitated laboratory strain that does not survive for extended periods, nor reproduce in the open environment. Even though some bacteria may survive the manufacturing process, results from controlled studies to detect viable cells on treated plants suggest that the cell production strain would not survive the common mixing and field application process for this biopesticide (refer to chapter on "Product Characterization").

Table 3a. Supplemental Studies for Expression in a Terrestrial Environment

Title of Study	Results	MRID No.
Environmental Fate and Degradation of Harpin	Harpin protein degraded rapidly on the surface of leaves within 3-4 days after application; and degraded quickly when reconstituted with pond water	445424-11
Detection of Endotoxin Contamination in Messenger; by D. W. Bauer, June 10, 1999	Amounts of endotoxin found in endproduct is lesser than amounts than that found in potable water	448702-14

Analysis of Residual Antimicrobial Activity in Messenger; by C. Lee-Tataseo and D. Bauer, June 30, 1999	Streptomycin used in fermentation to produce Messenger is not present at levels effective to inhibit growth of two bacterial species, *Pseudomonas aeruginosa* and *Rhodotorula glutinis*	448702-15
Analysis of Potential Resistance Genes from Messenger; by C. Lee-Tataseo and D. Bauer, June 17, 1999	The potential for transfer of the antibiotic resistance to ampicillin, streptomycin and spectinomycin by other microorganisms was tested by transformation of *E. coli* cells, and by conjugation with a marked *E. coli* strain. There were no instances of transfer by transformation or conjugation.	448702-16
Survival of Messenger Product Cell Line on Plant Surfaces and In Soil	The level of transformed *E. coli* K-12 in the endproduct will not significantly add to the level of antibiotic-resistant bacteria, or to bacteria in general in the environment. Those transformed *E. coli* K-12 cells that may survive the manufacture and preparation of the endproduct will die off within hours to 8 days after application, rather than reproduce in the environment.	449537-01

4. Ecological Exposure and Risk Characterization

A potential for exposure exists for Messenger to all terrestrial nontarget organisms because of the foliar use patterns. However, the nontarget data base to date (Table 3), indicates that Messenger® is practically nontoxic to avian, freshwater fish and aquatic invertebrates, plant seedlings, and freshwater algae. Based on the results of the honeybee study, harpin protein would be classified as practically non-toxic to honeybees, relative to the positive control dimethoate. Depending on the agricultural use site and IPM practices, estimated environmental concentrations (EEC values) would range from a low of 2 ppm harpin/A (= 2.25 oz. Messenger in a 25-gallon application) to a maximum of 12 ppm harpin/A (= 13.35 oz Messenger in a 25 gallon application). BPPD believes that intended exposures of harpin to honeybee populations will not approach the maximum EEC in the majority of cases. Finally, any transformed *E. coli* which survive the manufacturing process are unlikely to pose concerns for adverse risks to nontarget organisms and to the environment.

IV. PUBLIC INTEREST FINDING

Harpin (Messenger®) confers systemic resistance to many diseases, reduces infestations of selected insects, and enhances the growth, general vigor, and yield of a broad range of major and minor crops including fruits, vegetables, traditional agronomic crops, trees and ornamentals.

The broad efficacy, multiple benefits, and environmental compatibility of Messenger make it viable alternative to many chemical pesticides. In five large-scale tomato field trials in Florida, Messenger IPM programs decreased chemical usage an average of 71%, with a corresponding control of disease at least as effective as conventional fungicides. A reduction in pesticide usage of this magnitude would have a significant positive impact on residue levels, worker exposure, non-target insect suppression, resistance management, water quality and grower economics. Applied at rates of only 2-11.5 grams of active ingredient per acre, the product degrades rapidly in the environment after application, thus posing little or no concern as a ground and surface water contaminant. Additionally, low use rates and rapid degradation results in negligible residue on treated crops and minimal exposure to humans and livestock via food consumption. Messenger has been shown to possess an extremely low level of toxicity to mammals, with results of acute toxicity studies placing the product in Toxicity Category IV. Likewise, acute toxicity studies on non-target organisms (bobwhite quail, trout and honeybee) have yielded no adverse effects.

Harpin (Messenger®) has been shown to be effective against certain viral diseases, for which there are no current controls or resistant varieties, for example tobacco and cucumber mosaic viruses (TMV and CMV) in tomato and pepper, TMV in tobacco, and beet curly-top virus in jalapeno peppers, resulting in yield increases. Messenger has also proven effective against soil-borne pathogens and pests such as certain nematodes and *Fusarium*, which have few effective controls, except for methyl bromide. It also controls diseases such as bacterial wilt of tomato (*Pseudomonas solanacearum*), tomato and pepper rot (*Phytophthora capsici*) and bacterial blight of geranium (*Xanthomonas campestris vesicatoria*) for which there are currently no effective products.

Messenger exhibits no direct antimicrobial, inhibitory, or toxic activities toward plant pathogens or other organisms. Instead, the product confers systemic acquired immunity to the plant, thereby promoting optimum plant health, increased growth, vigor, yield, and quality. Because Messenger exerts no direct inhibitory effects on target pests and pathogens, it limits selection pressure for pest resistance development, and will provide growers with an important resistance management tool. Therefore, we find that it is in the public interest to grant a conditional registration for Harpin (Messenger®).

V. RISK MANAGEMENT AND REGISTRATION DECISION

A. DETERMINATION OF ELIGIBILITY

Section 3(c)(5) of FIFRA provides for the registration of a new active ingredient if it is determined that (1) it will not generally cause unreasonable adverse effects on the environment when use in accordance to widespread and commonly recognized practices and (2) its labeling and other materials required to be submitted comply with the requirements of FIFRA.

To satisfy criterion (1) above, it is believed that this biochemical pesticide will no cause any unreasonable adverse effects on human health or the environment given its low use rates,

lack of demonstrable toxicity, and instability in the environment. In addition, all data and labeling requirements have been fulfilled and found acceptable, thereby satisfying criterion (2).

Therefore, Harpin protein is eligible for registration under FIFRA Section 3(c)(5). The registered uses are presented in Table 4 of Appendix A.

B. REGULATORY POSITION

1. Registration

Data submitted are sufficient for the registration under Section 3(c)(5) of FIFRA of Harpin (Messenger®) for the use patterns presented in Table 4, Appendix A.

2. Tolerance Exemption

The Agency published in the Federal Register on May 3, 2000 (65 FR 25660) a final rule exempting residues of harpin protein from the requirement of a tolerance.

3. CODEX Harmonization

There is currently no CODEX Maximum Residue Limit set for food use of this active ingredient.

4. Non-Food Registration

The registered uses for Harpin protein include turf, trees and ornamentals, in addition to food uses.

5. Risk Mitigation

Because Harpin protein is classified in Toxicity Category IV, minimal personal protective equipment (PPE) is required to be worn by pesticide applicators and handlers i.e., long-sleeved shirt and long pants; shoes and socks; and dust mask. In addition, a Restricted Entry Interval (REI) of 4 hours is required to mitigate potential occupational exposure.

6. Endangered Species Statement

There are no expected toxic effects on non-target species based on toxicity and residue data. Therefore, the Agency has determined that this action will have no effect on listed species.

C. LABELING RATIONALE

1. Human Health Hazard

(a) Worker Protection Standard

Any product whose labeling reasonably permits use in the production of an agricultural plant on any farm, forest, nursery, or greenhouse must comply with the labeling requirements of PR Notice 93-7, "Labeling Revisions required by the Worker Protection Standard (WPS), and PR Notice 93-11, "Supplemental Guidance for PR Notice 93-7, which reflect the requirements of EPA"s labeling regulations for worker protection statements (40 CFR part 156, subpart K). These labeling revisions are necessary to implement the Worker Protection Standard for Agricultural Pesticides (40 CFR part 170) and must be completed in accordance with, and within the deadlines specified in PR Notices 93-7 and 93-11. Unless otherwise specifically directed, all statements required by PR Notices 93-7 and 93-11 are to be on the product label exactly as instructed in those Notices.

After April 21, 1994, except as provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR Notice complying labeling when they are distributed or sold by the primary registrant or any supplemental registered distributor.

After October 23, 1995, except as provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR Notice complying labeling when they are distributed or sold by any person.

The labels and labeling of all products must comply with EPA's current regulations and requirements as specified in 40 CFR 156.10 and other applicable notices. Labeling must also conform to Worker Protection Safety standards where re-entry into sprayed fields must not take place until sprays have dried unless protective clothing is employed.

b. Non-Worker Protection Standard

There are no current use sites for Harpin that are not covered by the Worker Protection Standard.

c. Precautionary Labeling

Since the End-Use Product is in Toxicity Category IV, no human health precautionary statements are required.

d. Spray Drift Advisory

Since Harpin may be applied with conventional aerial equipment, the following language will be required:

SPRAY DRIFT FOR AERIAL APPLICATION

Avoiding spray drift at the application site is the responsibility of the applicator. The interaction of many equipment-and-weather-related factors determine the potential for spray drift. The applicator and the grower are responsible for considering all these factors when making decisions.

2. Environmental Hazards Labeling

a. End-Use Product Environmental Hazards

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment washwaters.

b. Manufacturing-Use Product Environmental Hazards

There is currently no manufacturing-use product registered for Harpin.

D. LABELING

1. End-Use Product Name: MESSENGER
Active Ingredient:
Harpin Protein 3.0%
Other Ingredients 97.0%
Total.....100.0%

The Product labels shall contain the following information:

- Product Name
- Ingredient Statement
- Registration Number
- "Keep Out of Reach of Children"
- Signal Word "CAUTION'

VI. ACTIONS REQUIRED BY REGISTRANT

All requirements for registration under FIFRA Section 3(c)(5) have been met and found acceptable.

VII. APPENDICES

A. USE SITES

Table 4. Registered Use Sites

Food Use Sites	**Official Date Registered**
All food commodities in agricultural fields and greenhouses	April 11, 2002
Non-Food Use Sites	
Trees, turf and ornamentals	

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