

256400
RECORD NO.

SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 12-26-89 OUT FEB 8 1990

FILE OR REG. NO. 55638-RN
PETITION OR EXP. PERMIT NO. _____
DATE OF SUBMISSION 11-29-89
DATE RECEIVED BY EFED 12-15-89
RD REQUESTED COMPLETION DATE 3-15-90 (Expedite to 2/12/90)
EEB ESTIMATED COMPLETION DATE 2-12-90
RD ACTION CODE/TYPE OF REVIEW 131

TYPE PRODUCT(S): I, D, H, F, N, R, S MPCA
DATA ACCESSION NO(S). 413086-8
PRODUCT MANAGER NO. Phil Hutton (17)
PRODUCT NAME(S) FOIL® Bioinsecticide - Transconjugant Bt EG 2424
containing anti-Coleopteran/Lepidopteran Exotoxin
COMPANY NAME Ecogen Inc.

SUBMISSION PURPOSE Section 3 Registration - submission of additional
nontarget insect data.

SHAUGHNESSEY NO. CHEMICAL, & FORMULATION S.A.I.

Lepidopteran active toxin 5.25 g

Coleopteran active toxin 2.25 g

1
200

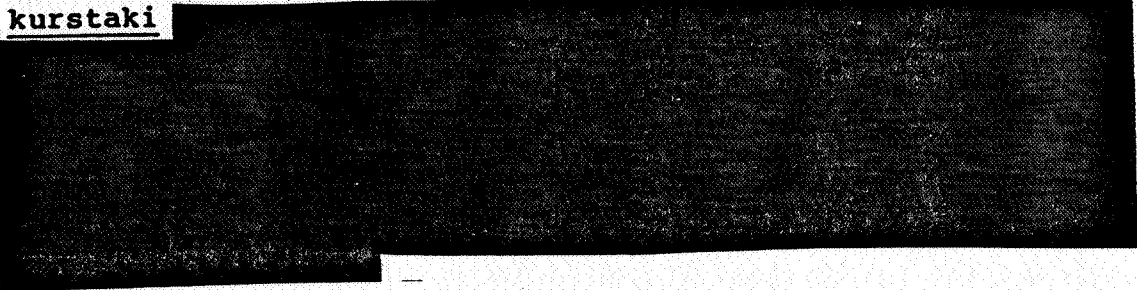
EEB REVIEW

Pesticide Name FOIL®-OF INSECTICIDE

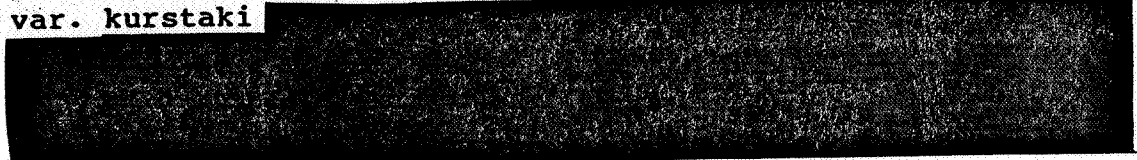
Bacillus thuringiensis var. kurstaki natural transconjugant EG 2424. (See October 24, 1986 Memo from A.E.Castillo to M.F.Galley of American Cyanamide Co., Agricultural Research Division: "Requirements and Conditions for the Amended Registration of B.thuringiensis", also section 101.1.0 of this review).

Company codes: EG 2424

EG 2424 is derived as follows: Bacillus thuringiensis var. kurstaki



Nontarget plant effects testing was performed with strain EG 2101 which is derived as follows. Bacillus thuringiensis var. kurstaki



MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

100.0.0 Submission Purpose and Label Information

100.1.0 Submission Purpose and Pesticide Use

Ecogen, Inc. has requested a Section 3 registration for an insecticide containing Ecogen's proprietary strain of B.thuringiensis var. kurstaki (EG 2424) as the active ingredient for control of the Colorado potato beetle, European corn borer, armyworms and loopers on potato, tomato and eggplant crops.

100.2.0 Formulation Information

FOIL®-OF (Oil Flowable Insecticide)

ACTIVE INGREDIENT:

<u>Bacillus thuringiensis var. kurstaki Strain EG2424:</u>	5.25 %
Lepidopteran active toxin.....	2.25%
Coleopteran active toxin.....	

INERT INGREDIENTS 92.5 %

Total 100.0 %

0.64 lbs a.i. per gallon

2
284

100.3.0 Application Methods, Directions, Rates

(From label)

Preharvest interval: There are no restrictions for applying FOIL OF up to the time of harvest.

FOIL®-OF for potatoes

Crop	Pest	Quarts/ Acre ^{1/}
Potatoes,	Colorado Potato Beetle ^{2/}	2 to 5
Tomatoes and Eggplant	European Corn Borer ^{3/}	2 to 5
	Armyworms and Loopers ^{4/}	2 to 5

- 1/ For ground applications use a minimum of 20 gallons of water per acre. For aerial applications use a minimum of 5 gallons of water per acre.
- 2/ Initial application should be made when 30% of observed egg masses have hatched. Subsequent sprays should be applied as needed.
- 3/ Initial application should be made at peak moth activity. Subsequent sprays should be applied weekly as needed.
- 4/ Treat when larvae are young (early instars) and are actively feeding on exposed plant parts. Apply before extensive foliar damage has occurred.

100.4.0 Target Organisms

Colorado Potato Beetle
European Corn Borer
Armyworms and Loopers

100.5.0 Precautionary Labeling

The label contains the following precautions:

CAUTION: Keep out of reach of children
Hazard to humans: Avoid inhalation or contact with eyes or skin

Reentry Statements, storage, disposal and container disposal directions are supplied.

3
2013

ENVIRONMENTAL HAZARDS:

Do not apply directly to water or wetlands (swamps, bogs, marshes and potholes). Do not contaminate water when disposing of equipment washwaters.

101.0.0 Hazard Assessment

101.1.0 Discussion

The Bacillus thuringiensis var. kurstaki strain used as a.i. in FOIL® is a transconjugant of naturally occurring Bacillus thuringiensis strains derived by natural plasmid exchange processes and not by recombinant DNA technology.

The rationale behind the new strain selection is as follows. The starting point is the observation that the plasmids carrying the toxin genes in individual isolates within a single subspecies may vary from one another. By screening Ecogen has identified a variety of wild type isolates with enhanced potencies against several target insect pests. Ecogen further enhanced the insecticidal activity by two approaches.

The first approach is partial plasmid curing where one or more of several toxin encoding plasmids are lost from the bacterium. The curing of plasmids is a spontaneous event and typically does not require induction (although it occurs more readily at elevated temperatures in some strains). By this process Ecogen produced strains only with demonstrated plasmid stability and subsequent uniformity in product potency. The second approach to strain improvement is by conjugal transfer. This is a natural process whereby mixing of two strains together results in the transfer of plasmids between strains.

Ecogen further showed that the above processes also occur in infected insects. It was demonstrated not only that conjugal transfer did occur in vivo, but that only those plasmids that could be mobilized in the previous in vitro studies were also mobilized in vivo.

The relative potencies of the transconjugant strains were also determined by comparison to the HD 1-S-1980 international standard. Strain EG 2424 produces only endotoxin. No beta exotoxin activity has been detected using a standard house fly bioassay.

The recombinant B.t. strain was field tested in 1987 and 1988 after a rigorous EPA review which included other government agencies and a SAP. Subsequent to the 1986

4
[Handwritten signature]

ENVIRONMENTAL HAZARDS:

Do not apply directly to water or wetlands (swamps, bogs, marshes and potholes). Do not contaminate water when disposing of equipment washwaters.

101.0.0 Hazard Assessment

101.1.0 Discussion

The Bacillus thuringiensis var. kurstaki strain used as a.i. in FOIL® is a transconjugant of naturally occurring Bacillus thuringiensis strains derived by natural plasmid exchange processes and not by recombinant DNA technology.

The rationale behind the new strain selection is as follows. The starting point is the observation that the plasmids carrying the toxin genes in individual isolates within a single subspecies may vary from one another. By screening Ecogen has identified a variety of wild type isolates with enhanced potencies against several target insect pests. Ecogen further enhanced the insecticidal activity by two approaches.

The first approach is partial plasmid curing where one or more of several toxin encoding plasmids are lost from the bacterium. The curing of plasmids is a spontaneous event and typically does not require induction (although it occurs more readily at elevated temperatures in some strains). By this process Ecogen produced strains only with demonstrated plasmid stability and subsequent uniformity in product potency. The second approach to strain improvement is by conjugal transfer. This is a natural process whereby mixing of two strains together results in the transfer of plasmids between strains.

Ecogen further showed that the above processes also occur in infected insects. It was demonstrated not only that conjugal transfer did occur in vivo, but that only those plasmids that could be mobilized in the previous in vitro studies, were also mobilized in vivo.

The relative potencies of the transconjugant strains were also determined by comparison to the HD 1-S-1980 international standard. Strain EG 2424 produces only endotoxin. No beta exotoxin activity has been detected using a standard house fly bioassay.

The recombinant B.t. strain was field tested in 1987 and 1988 after a rigorous EPA review which included other government agencies and an EPA Science Advisory Panel. Subsequent to the 1986 Ecogen notification, EPA accepted a database for nontarget testing performed with a

5
88

representative strain of Ecogen's group of transconjugant strains. (Oct. 24, 1986 letter from A.E. Castillo to M.W. Galley). EPA did, however, require Ecogen to submit nontarget beneficial predatory insect and honeybee toxicity data for each new strain to be registered.

Anti-coleopteran active strains had not been developed at the time of this agreement. Ecogen, therefore, was asked to develop a separate set of nontarget data for strains containing the anticoleopteran endotoxin.

A review of the studies submitted with this application shows that there is some intrinsic toxicity to aquatic invertebrates and some nontarget insects at maximum hazard doses. However no significant acute risk to nontarget wildlife is expected from exposure to FOIL® at the proposed label use rates.

101.2.0 Likelihood of Adverse Effects to Nontarget Organisms

a. Avian Studies

When administered by oral gavage at 3.33 gm/kg of body weight EG 2424 had no apparent effect upon mallard ducks for 30 days. The acute pathogenicity LD₅₀ value to mallards by gavage was determined to be greater than 3.33 gm/kg of body weight.

When administered by oral gavage at 3.33 gm/kg of body weight EG 2424 had no apparent effect upon bobwhite quail for 30 days. The acute pathogenicity LD₅₀ value to bobwhite by gavage was determined to be greater than 3.33 gm/kg of body weight.

In view of the lack of oral toxicity, no significant avian hazard is expected from exposure to FOIL® insecticide at the proposed label use rates.

b. Fish Studies

A 30 day static renewal toxicity and pathogenicity study of EG 2424 was conducted with rainbow trout. Thirty fish were exposed to a maximum hazard dose of 1×10^6 spores/ml of water and 1×10^8 spores/mg in food. No toxic or pathogenic responses were noted. At necropsy (14 and 30 days) internal organs were found normal in appearance. Upon culture some organs were found to harbor the MPCA. No systemic blood involvement, however, was apparent.

In view of the above results, no significant freshwater fish hazard is expected from exposure to FOIL® insecticide at the proposed label use rates.

6
[Handwritten signature]

c. Aquatic Invertebrate Studies

A 21 day static renewal toxicity and pathogenicity study of EG 2424 was conducted with Daphnia magna. Forty daphnids were tested for each of three concentration levels, including a maximum hazard dose of 1×10^6 spores/ml of water. Statistical analysis of survival for Daphnia magna in treated levels showed a difference in survival in the 1.1×10^6 concentration from that of untreated controls. A 21 day EC₅₀ was calculated to be 9.5×10^5 spores/ml. The point estimate for the MATC value was 8.1×10^5 spores/ml.

In view of the terrestrial uses of the insecticide the exposure to aquatic organisms is expected to be several orders of magnitude lower than the MATC. Thus no freshwater aquatic invertebrate hazard is expected from the proposed uses of FOIL® insecticide.

d. Mammalian Wildlife

These studies are required only when toxicology data are inadequate for assessment of hazard to wild mammals.

The anticipated low exposure of mammalian wildlife during application indicates that risk to wild mammals from the proposed uses of FOIL® insecticide is minimal.

e. Estuarine and Marine Animal Studies

None submitted. These studies will need to be submitted prior to use of this product in or near estuarine or marine environments.

f. Nontarget Plant Studies

1. A 96 hour exposure to 1.47 mg/L of the typical end use product (TEP) (EG 2101-LX186-05 Oil Based Flowable) showed no reduction in the growth rate of the green alga Selenostrum capricornutum.

2. Vegetative vigor test (Tier II)

All 10 plant species tested with EG 2348-OF had a statistical no-effect level of 4.0 qt/a (0.622 lb. ai/a), the highest concentration tested, in phytotoxicity ratings and plant height. All plant species had a no-effect level for plant dry weight of 4.0 qt/a except lettuce which had a no-effect level of 2.0 qt/a. Lettuce dry weight was the only crop/parameter to exhibit a dose response. Probit analysis showed EC₂₅ and EC₅₀ values of 51.4 and 61211 qt/a respectively.

7

3. Seed germination/emergence

Seed germination and seedling emergence studies were performed on 10 plant species using the maximum label rate of 2.0 qt/a (0.311 lb ai/a) of EG 2348-OF. The seed germination study showed no statistically significant ($p < 0.05$) effect on radicle length or the percentage of seeds germinating, regardless of plant species. The seedling emergence study showed that EG 2348-OF did not result in a greater than 25% detrimental effect on seedling height, percentage of seedlings emerged or plant dry weight.

In view of the above results no nontarget plant hazard is expected from the proposed uses of FOIL® insecticide.

g. Honey Bee Studies

A 4 day bell-jar dusting chamber study with honey bees exposed to approximately 25 mcgm/bee of 100% a.i. EG 2424 (equiv. to 25 lb/A application rate) showed no apparent detrimental effects.

In view of the above results, no significant acute contact toxicity to honey bees is expected from exposure to FOIL® insecticide at the proposed label use rates. It is not possible to determine any chronic effects from repeat applications without dietary studies of longer duration.

h. Nontarget Beneficial Insect Studies

1. Ladybird beetles: the 48-hr acute contact toxicity LD₅₀ with 100% a.i. (EG 2424) was >0.56 mg/beetle.
2. Parasitic hymenoptera: (*Brachymeria intermedia*):

The 48-hr acute contact toxicity LD₅₀ with 100% a.i. (EG 2424) was >0.56 mg/insect. The mortality was 28% at 0.56 mg, the highest dose tested and may have been treatment related.

A 30 day dietary toxicity/pathogenicity LC₅₀ was determined to be greater than 250 mcgm/ml feed. The mortality at 30 days in the 250 mcgm/ml treatment group was 22% showing possible chronic toxicity at maximum hazard dose levels.

3. Green Lacewing larvae: A 92 hr. bell-jar dusting chamber study with larvae exposed to approximately 20 mcg/larva of 100% a.i. EG 2424 (equiv. to 20 lb/A application rate) showed no apparent adverse effects.

8
224

In view of the above results, no significant acute contact toxicity to predatory beneficial insects is expected from exposure to FOIL® insecticide at the proposed label use rates. It is not possible to determine any chronic effects to lady beetles or green lacewing larvae from repeat applications without dietary studies of longer duration.

101.3.0 Endangered Species Considerations

Based on the toxicity and exposure data, EEB feels that there will not be a "may affect" situation for endangered mammals, birds, plants and aquatic species.

Ecogen Inc. agrees that endangered insect species restrictive labeling will be implemented when such labeling is deemed necessary based on data received from the Bacillus thuringiensis Reregistration and/or further consultation with the Fish and Wildlife Service, Department of the Interior.

101.4.0 Adequacy of Toxicity Data

The registrant has addressed all of the data requirements outlined in the Pesticide Assessment Guidelines, Subpart M. Some of the studies are of the the supplemental category because they were initiated prior to Subdivision M revision. Some nontarget insect testing, for example, was performed following subdivision L acute (short term) toxicity protocols. The studies submitted are, however, sufficient for assessing acute risk from the commercial use of naturally occurring delta endotoxins. They are not sufficient to fully characterize the intrinsic toxicity/pathogenicity to nontarget insects from chronic exposure to large doses of the active ingredient.

Plant studies performed with formulated EG 2348-OF (CONDOR®) are acceptable to fulfill the FOIL® plant toxicity data requirements since the formulations of the two products are identical. The difference is in the a.i. Bacillus thuringiensis strains, none of which are associated with plant pest problems. EG 2348-OF studies, therefore, should reflect any phytotoxicity associated with FOIL® insecticide formulations.

101.5.0 Adequacy of Labeling

The precautionary labeling (see sec. 100.5.0) is adequately prepared.

102.0.0 Classification N/A

9
223

103.0.0 Conclusions

EEB has reviewed the Section 3 Registration application of FOIL®-OF (7.5 % Oil Flowable Insecticide) for control of Colorado potato beetle, European corn borer, armyworms and loopers on potatoes, tomatoes and eggplant by aerial and ground application. EEB concludes that acute toxicity risk to nontarget wildlife from the use of FOIL® is minimal at the proposed label use rates.

EEB requests that Ecogen develop chronic toxicity/pathogenicity data on honey bees and ladybird beetles for complete assessment of the long term effects on beneficial nontarget insect populations from prolonged use of this product (see Sec. 101.4.0).

Zig Vaituzis, Microbiologist
Ecological Effects Branch (H7507-C)
Environmental Fate and Effects Division

Zig Vaituzis 2/9/90

Ray Matheny, Head, Section 1
Ecological Effects Branch (H7507-C)
Environmental Fate and Effects Division

Ray W. Matheny 2/9/90

fo

James W. Akerman, Branch Chief
Ecological Effects Branch (H7507-C)
Environmental Fate and Effects Division

Ray W. Matheny 2/9/90