UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

007368

JUL 27 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Bacillus thuringiensis transconjugant strain EG2424 (Foil OF Insecticide). Ecogen, Inc. Registration Application for Use on Potatoes (I.D. No. 55638-RN; Record No. 237327; MRID No. 40951101; Caswell No. 66; HED Project No. 9-0631)

TO: Phil Hutton/Mike Mendelsohn (PM-17)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: William J. Hazel, Ph.D., Chemist/Plant Pathologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

W. J. Hazel 5/5/89

and

Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination Branch
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R. D. Sjoblad 7/25/89

THRU: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Reto Engler

Introduction

Ecogen, Inc. has submitted an application to register a 7.5% oil flowable (OF) formulation of their Bacillus thuringiensis (Bt) transconjugant strain EG2424, Foil OF Insecticide, for use on potatoes. This strain was field tested in 1988 under an Experimental Use Permit (I.D. No. 55638-EUP-3). Deficiencies requiring satisfaction prior to Section 3 registration were identified in the 5/30/88 W. Hazel review of the EUP request. Transconjugant Bt strains, such as EG2424, are also subject to the data requirements listed in the Bt Registration Standard, issued 12/88. As a result, certain requirements may have changed or been added. To avoid repetition, only unresolved issues, unsatisfied requirements, and data not reviewed in the 5/30/88 memorandum will be discussed here.

Product Identity

151A-10. Product identity and disclosure of ingredients. The composition of the end-use product (EP) has been altered somewhat

(see identity of the plasmid donors and modifications to the Confidential Statement of Formula, CSF, in the Confidential Appendix). Of the eight key identification criteria listed in the Registration Standard, the H-antigen serotyping, strain history, plasmid profiles, crystal inclusion description, and identity of insecticidal toxins produced have been adequately defined. The following additional data are required:

- Standard biochemical testing according to Vol. 2 of Bergey's Manual of Systematic Bacteriology. Several laboratories may be contracted to perform such tests rapidly and economically.

- Standard Gram-positive antibiotic (including erythromycin) sensitivity testing.

- Although representatives of the insect orders Coleoptera, Hymenoptera, and Lepidoptera have been tested for their sensitivity to EG2424, a representative of each of the following orders must also be tested: Diptera, Orthoptera, and Trichoptera.

- Although it is true that data reflecting subcutaneous injection of mice with each batch must be conducted (yet not submitted), such a study must be submitted on one representative batch as a condition of registration.

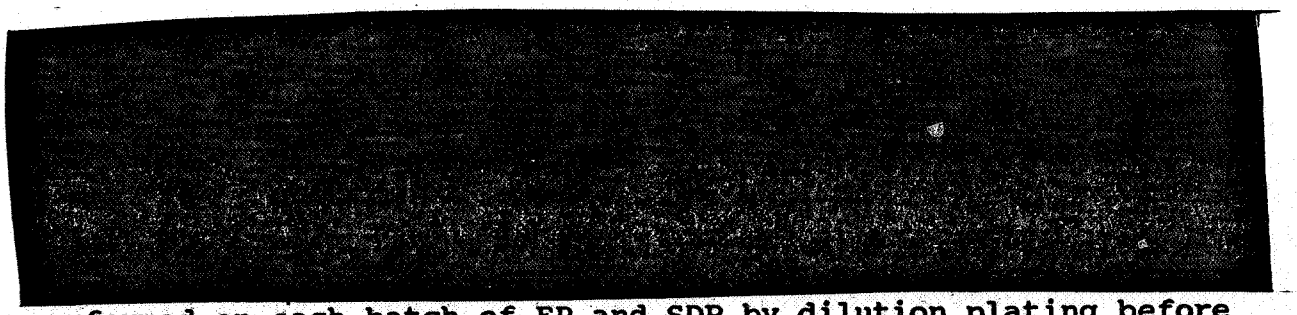
- If the FBC or analogous manufacturing-use product (MP) is to be sold (there is evidence that it will at least be transported), then the MP product must be registered and a CSF submitted bearing nominal concentrations and certified limits for the ai, impurities of potential toxicological concern, and each intentionally-added inert. Ecogen must specify whether an MP or equivalent is to be sold or if the EP will be formulated at an Ecogen-owned or -contracted installation.

151A-11. Manufacturing process. The manufacturing process has not been significantly altered since the time of the EUP submission. All of the deficiencies cited in the 5/30/88 W. Hazel memorandum requiring satisfaction prior to Section 3 registration have been adequately addressed/fulfilled. No deficiencies remain.

151A-12. Discussion of the formation of unintentional ingredients. All weaknesses cited in the 5/30/88 review have been resolved. No deficiencies remain.

151A-13. Analysis of samples. [redacted]

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED



performed on each batch of EP and SDP by dilution plating before and after heat treatment (65 C for 30 min), respectively (no data provided).

The available data largely satisfy the requirements of this section. However, the following are required:

- Although SACB agrees that spore counts need not appear on the label, the viable spore count of five batches must be submitted as a practical range.

- The toxins in Foil OF and the MP must be analyzed such that insect order bioactivity can be determined eg., %(w:w) lepidopteran-active toxin(s) and %(w:w) coleopteran-active toxin(s). The ingredient statement of the EP label must reflect this as noted on p. 22 of the 12/88 Registration Standard.

151A-15. Certification of limits. Ecogen certifies that the total delta-endotoxin content (sum of one or more of both P1- and Cry C-type toxins) of Foil OF will be from 6-9.0% (w:w), reflecting batch and analytical variability. Upper limits were proposed for contaminating microbes in the EP and SDP. Since endotoxins active against more than one insect order are present in Foil OF, the label and CSF should state this and the following must be provided:

- Certified upper and lower limits for each group of endotoxins distinguished by insect order affected. Validated methods to enforce such limits must be submitted.

151A-16. Physical and chemical properties. Only noteworthy changes/additions since the EUP submission will be presented:

Density - EP, 8.00 lb/gal

Stability - EP shown, by bioassay, not to lose activity upon exposure to an electrolyte solution of nine cations (5-70 ppm) for 24 hr at room temperature and pH 7.0. Biological activity against both a lepidopteran and a coleopteran test species tended to decline as pH increased from 5 to 9 within 48 hr at 20 C.

Storage stability - EP and MP, no significant loss in endotoxin concentration after 6 mo at 20 C. Data at the 12-mo interval should be available 4/89.

Miscibility - Neither the MP nor the EP is emulsifiable.

Corrosiveness - A reasonable argument supporting a lack of corrosiveness to the commercial containers was presented for purposes of a conditional registration.

The submitted data largely satisfy the requirements for physical and chemical characteristics. Once available, the following data should be submitted:

- Storage stability data after storage of EP and MP for 1 year. The analytical method used to determine endotoxin should be specified. Also, any corrosion of containers, or lack thereof, should be noted.

Residue Chemistry

Tolerances. Ecogen has stated that they have provided data/information demonstrating that B. thuringiensis EG2424 falls under the tolerance exemption at 40 CFR 180.1011 (Section F not provided to SACB). Although this appears to be the case, registration of products containing EG2424 is contingent upon satisfaction of product chemistry and toxicology data requirements.

Proposed use directions. The 7.5% OF is proposed for use at 1-4 qt product/A to control Colorado potato beetle, European corn borer, armyworms, and loopers on potatoes. Aerial or ground applications are to be made when larvae are young and actively feeding at 5- to 10-day intervals as needed. A sticker may be used. The following amendments should be made to the OF label:

- The active ingredient statement must be presented as outlined on p. 22 of the 12/88 Bt Registration Standard, i.e., toxins subdivided by insect order affected.

cc: F. Betz (SACS, EFED), Z. Vaituzis (EEB, EFED), R. Pilsucki (EFGWB, EFED)

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CONFIDENTIAL APPENDIX

Ecogen's Foil OF

HED Project No. 9-0631

Hazel/Sjoblad (SACB)

1 page

MAMMALIAN TOXICOLOGY

007368

Introduction.

Ecogen, Inc. has submitted the following mammalian toxicology studies to support the registration of Foil OF insecticide. As per agreement between R. Engler (HED) and P. Hutton (RD), the studies were reviewed by M. Mendelsohn (RD), with secondary review being provided by R. Sjoblad (HED).

The submitted studies are summarized as follows:

§152A-10. Acute oral toxicity/pathogenicity.

An unacceptable acute oral toxicity/pathogenicity study was submitted. A diluted preparation of the test material (1:30 dilution) was not toxic to or pathogenic for rats. Greater than 10^4 colony-forming units of the active bacterial ingredient were isolated from fecal material at day 1 after dosing, but not from any organ/body fluid/fecal material at any other time during the study. The study can be upgrade to acceptable to support registration of the active ingredient (but not the end-use product) if the method used to isolate the active bacterial ingredient from the test animals is described, and if an adequate explanation is forwarded on why body weights of certain test animals were not recorded at necropsy.

§152A-11. Acute dermal toxicity.

An unacceptable acute dermal toxicity study was submitted. A diluted preparation (1:30 dilution in saline) was slightly toxic (Tox category IV) to rabbits when applied at 1 ml for 24 hours. The study is unacceptable because a diluted preparation of test material was used.

§152A-12. Acute pulmonary toxicity/pathogenicity.

The submitted acute pulmonary toxicity/pathogenicity study was unacceptable because the data presented were not adequate to establish a pattern of clearance of the test bacterium from test animals, the method used for enumeration of the active bacterial ingredient from test animals was not described, and body weight changes of certain animals were not reported. The test material when administered to rats via intratracheal instillation, at 9.5×10^8 CFU/animal was lethally toxic to 9/15 females and 4/15 males. Necropsy of dead animals showed a high incidence of hemorrhage in the lungs.

§152A-13. Acute intravenous toxicity/pathogenicity.

An unacceptable acute intravenous toxicity/pathogenicity study in rats was submitted. The data showed that the active bacterial ingredient at approximately 8×10^7 CFU/animal was not toxic to or pathogenic for test animals after intravenous injection. The data showed that the active bacterial ingredient is slowly cleared from the test animals. The study can be upgrade to acceptable if the method used to isolate the active bacterial ingredient from the test animals is described, levels of the test bacterium from blood at 1 hour post-dosing are

reported, and an acceptable explanation for lack of reporting of certain body weight data is forwarded.

§152A-14. Primary eye irritation.

The primary eye irritation study submitted was unacceptable because a 1:30 dilution (in saline) of the basic formulation was used. Thus, the potential for the end-use product to cause eye irritation cannot be determined. The dosing material, under the conditions of the study falls in Tox category III. Since goggles are to be worn by applicators, mixers, and loaders, this study may not have to be repeated.

The Data Evaluation Reports for these studies are attached.

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Reviewed by: Michael Mendelsohn, Microbiologist
PM Team 17, IRB, RD (H7505C) Michael Mendelsohn
Secondary Reviewer: Roy D. Sjoblad, Ph.D., Microbiologist RDS
SACB, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity/pathogenicity study in the rat.

MRID NO.: 409511-02 Caswell No.: 66G

TEST MATERIAL: Foil OF Insecticide Basic Formulation Containing 2.38 x 10^{10} CFU *B.t.* subsp. *kurstaki* strain EG2424/ml

SYNONYMS: *Bacillus thuringiensis* transconjugant

STUDY NUMBER: G-7138.222

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute oral toxicity/pathogenicity study of Foil OF Insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: November 23, 1988

CONCLUSIONS: The study showed a lack of toxicity/pathogenicity of a 1/30 dilution of Foil OF Insecticide Basic Formulation. The number of Colony Forming Units (CFUs) of strain EG2424 (7.93 x 10^{8} CFU strain EG2424/ml) were adequate in evaluating the acute oral toxicity of the active ingredient. However, this dilution does not adequately support the end-use product.

CLASSIFICATION: Unacceptable. Sections B and E of the discussion need to be resolved before this study can be considered acceptable.

I. STUDY DESIGN: A. Test Material: Foil OF Insecticide Basic Formulation assigned MBA chemical No. T07138A; received June 8, 1988; Containing 2.38 x 10^{10} CFU *B.t.* subsp. *kurstaki* strain

EG2424/ml (per Ecogen letters dated 4/18/89 and 4/24/89 from Dave Olson to Mike Mendelsohn).

B. Test Animals: young adult (approximately 6-7 weeks old at dosing) male and female Sprague Dawley rats; from Harlan Sprague Dawley, Inc., Frederick, MD; Weight: 178-226 g (males) and 112-138 g (females) on day of dosing.

C. Methods: Thirteen male and thirteen female rats each were dosed orally via feeding needle with 1.0 ml of a 1/30 dilution of the test material containing 7.93 x 10^8 CFU B.t. subsp. kurstaki strain EG2424/ml. Animals were fasted overnight prior to dosing and also for up to 4 hours after dosing. An untreated control group consisted of 13 male and 13 female rats. Animals were observed for clinical signs of toxicity/disease at one hour after dosing, and then once per day for the 20 days post-treatment or until interim sacrifice. Individual animal body weights were determined at the time of dosing and the weekly during the study. Two male and two female rats from the dosed group and one female and one male from the untreated group were sacrificed at 1, 7, 14,and 21 days after dosing for evaluating infectivity. The cardiac blood, kidneys, brain, liver, lungs, spleen, mesenteric lymph nodes, and fecal samples from these animals were analyzed for the presence of the test bacterium. All animals surviving to study termination (i.e., 21 days after dosing) were necropsied. In addition, the effects of heating on the infectivity of the test microbe was investigated by heating samples of tissue (unspecified except for lung and feces) exposed to the test microbe to 56 degrees C and comparing plate counts of heated verses non-heated tissue or feces.

II. RESULTS: The test material had no significant effect on body weight gain by test animals; all treated animals gained weight during the study, and mean body weight gain values were similar among treated and control groups (although body weight gain for treated was slightly higher than for the control groups). No test-substance related lesions were observed in any test substance at the 21-day terminal sacrifice time. No CFU of the active bacterial ingredient were detected on growth media upon plating homogenates of tissue/organ from any control or dosed animals. Greater than 10^4 CFU EG2424/g feces was determined at day 1. Thereafter EG2424 was not found in fecal samples. With regard to heating effects, an unheated sample of lung tissue produced approximately six colonies per plate while a

heated sample produced none. An unheated sample of fecal tissue produced roughly eight times as many colonies as did the heated sample.

III. DISCUSSION:

A. The appropriate dosing material to be used in this acute oral study would have been the undiluted Foil OF Insecticide formulation to be used in commerce.

B. The recovery method that was used to select for strain EG2424 from tissue homogenates, blood, and feces is unclear. This must be specified.

C. It is unclear how the effects of heating on the infectivity of the test microbe was investigated and how generated data would be applicable towards understanding the potential infectivity of the test organism. Does 56 degrees C kill spores of the test organism or was the purpose of heating to compare the effects of a spore vs. a spore/vegetative cell preparation? What and how many tissues were tested? How were the tissues exposed to EG2424? How long were the samples heated? Were there controls?

D. The methods used to estimate the units of bacteria in the test sample and date of analysis should accompany the study report. Where possible, the estimate of bacterial units should be done prior to dosing of the test animals.

E. It is unclear why body weights were not determined for animals 2602, 2615-2618, 2628, and 2641-2644 on the date of necropsy. This should be explained.

Reviewed by: Michael Mendelsohn, Microbiologist
PM Team 17, IRB, RD (H7505C) Michael Mendelsohn
Secondary Reviewer: Roy D. Sjoblad, Ph.D., Microbiologist
SACB, HED (H7509C) RDS

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity in Rabbits

MRID NO.: 409511-03 Caswell No.: 66G

TEST MATERIAL: Foil OF Insecticide Basic Formulation Containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7138.232

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: EPA/OECD Acute Dermal Toxicity Study of Foil OF Insecticide in Albino Rabbits

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: November 21, 1988

CONCLUSIONS: A 1/30 diluted Foil OF Basic Formulation was slightly toxic (Tox Category IV) when applied at 1 ml to the skin of rabbits.

CLASSIFICATION: Unacceptable; because diluted test material was used.

I. STUDY DESIGN: A. Test Material: Foil OF Insecticide Basic Formulation assigned MBA chemical No. T07138 (lot number 178-31); received June 8, 1988; containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml (per Ecogen letters dated 4/18/89 and 4/24/89 from Dave Olson to Mike Mendelsohn).

B. Test Animals: young adult (approximately 10 weeks old at dosing) male and female New Zealand albino rabbits, from Hazelton Research Products, Denver, PA; Weight: 2.1-2.4 kg on day of dosing.

C. Methods: At 24 h before dosing, the test material was applied to the shaved trunks of 5 male and 5 female animals. The dosing material was a preparation of the test article which had been diluted in saline (1 ml of the test article in 29 ml saline) to give a bacterial concentration of 7.9 x 10^{8} CFU/ml. One ml was applied to the skin of each test animal. The treated areas were covered with gauze and tape, and animal midsections were then wrapped with cloth toweling, secured to the animals with tape. At 24 hours after dosing all coverings were removed, and excess test material was removed by wiping. Animals were observed for clinical signs of toxicity three times on the day of dosing, and then once per day for 14 days. Individual animal body weights were determined at the time of dosing and then weekly during the study. All animals surviving to study termination (i.e., to 14 days after dosing) were necropsied.

II. RESULTS: All animals survived the 14 day observation period. Seven animals had increased respiration on Test Day 2; however on Test Day 3 all signs of respiratory distress had ceased. Slight to moderate erythema was observed in three animals on Test Day 3 but not after. All animals gained weight during the study and gross necropsies performed showed no "treatment-related" lesions.

III. DISCUSSION:

A. The dosing material should have consisted of undiluted test article material. Lesions during necropsy should have been noted whether thought to be treatment-related or not.

B. The specific animals showing clinical signs in Test Day 2 and 3 should have been noted. They were not noted.

C. The 1/30 dilution of the Foil Of Basic Formulation falls in Toxicity Category IV for Skin Effects.

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Reviewed by: Michael Mendelsohn, Microbiologist
PM Team 17, IRB, RD (H7505C) Michael Mendelsohn
Secondary Reviewer: Roy D. Sjoblad, Ph.D., Microbiologist RDS
SACB, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute pulmonary toxicity/pathogenicity study in the rat.

MRID NO.: 409511-04 Caswell No.: 66G

TEST MATERIAL: Foil OF Insecticide Basic Formulation Containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7138.225

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute pulmonary toxicity/pathogenicity study of Foil OF Insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: November 23, 1988

CONCLUSIONS: The infectivity/clearance of strain EG2424 is unclear due to the high incidence of mortality (9/15 females, 5/15 males) and apparent inconsistencies with regards to the presence of EG2424 in lung tissue when 4.52 x 10^{8} CFU strain EG2424 were applied per animal via intratracheal instillation.

CLASSIFICATION: Unacceptable. Section A, C, and E of the discussion need resolution before this study can be considered acceptable.

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I. STUDY DESIGN: A. Test Material: Foil OF Insecticide Basic Formulation assigned MBA chemical No. T07138A; received June 8, 1988; containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml (per Ecogen letters dated 4/18/89 and 4/24/89 from Dave Olson to Mike Mendelsohn).

B. Test Animals: young adult (approximately 6-7 weeks old at dosing) male and female Sprague Dawley rats; from Harlan Sprague Dawley, Inc., Frederick, MD; Weight: 200-236 g (males) and 119-145 g (females) on day of dosing.

C. Methods: Fifteen male and fifteen female rats each were dosed with 0.04 ml of the test material via intratracheal instillation (9.52 x 10^{8} CFU strain EG2424/animal). An untreated control group consisted of 15 male and 15 female rats. Animals were observed for clinical signs of toxicity/disease at 2.5 hours after dosing, and then once per day for 20 days post-treatment or until interim sacrifice. Individual animal body weights were determined at the time of dosing and then weekly during the study. Two male and two female rats from the dosed group and one female and one male from the untreated group were sacrificed 1 and at 7 days after dosing for evaluating infectivity/clearance. Due to high incidence of mortality among the female treatment group (9/15), this procedure was modified on 14 and 21 days after dosing. On these days, one control animal per sex and two male treatment group animals were sacrificed for infectivity/clearance determinations. Blood, kidneys, brain, liver, lungs, spleen, mesenteric lymph nodes, from these animals were analyzed for the presence of the test bacterium. All animals surviving to study termination (i.e., 21 days after dosing) and those which died during the study were necropsied. In addition, the effects of heating on the numbers of the test bacteria was investigated by heating samples of tissue (unspecified except for lung) and feces exposed to the test microbe to 56 degrees C and comparing plate counts of heated versus non-heated samples.

II. RESULTS:

A. Body Weights: No data were reported on effects of the test article on body weight due to the high incidence of mortality.

B. Clinical Signs: Clinical signs included lethargy, and both rapid and shallow respirations. These signs were limited to study day 1. All affected animals died shortly after manifestation of the toxic signs or recovered and appeared normal until time of scheduled sacrifice. Five of the male treatment group (3 found dead on the day of dosing, 1 accidental death on the day of dosing, and 1 death 1 day after dosing) and nine of the female treatment group died (8 found dead on the day of dosing. and 1 found dead 1 day after dosing) after manifestation of toxic signs. It is not clear from the report whether deaths on the day of dosing occurred immediately after dosing or between dosing and 2.5 hours after dosing. Thirteen males and thirteen females were lethargic; rapid respirations were seen in six treated males and ten treated females; shallow breathing was observed in five treated males and ten treated females. No clinical signs of toxicity were observed in any control group animals.

C. Infectivity/Clearance Determinations: The presence of the microbe was limited to the lungs of both male and female treatment groups. In the males, the presence of the microbe was observed on treatment day 14 (no colonies were found on the plates from days 1 to 7) and the microbe was cleared by day 21. In females, no EG2424 was found on day 1, but lung homogenates plated on day 7 showed the presence of the test microbe. Clearance data in not available, however, because of the mortality of females after this time point.

On the day of dosing lung tissue homogenates were plated and the presence of the test microbe was observed in both males and females.

D. Necropsy:

FEMALES

Day of Dosing

On the day of dosing 8 treated females were found dead; 2 females exhibited extensive hemorrhage in all lung lobes, a pale yellow mass in the distal section of the trachea, and clear fluid being easily expressed from the lungs; 4 females exhibited extensive hemorrhage in all lung lobes.and clear fluid being easily expressed from the lungs; the remaining 2 females exhibited extensive hemorrhage in upper lung lobes, a pale yellow mass in the distal section of the trachea. and clear fluid being

easily expressed from the lungs. Two females were sacrificed on the day of dosing and showed red spots in all lung lobes.

One Day After Dosing

One day after dosing one female was found dead with the chest cavity being filled with clear fluid and extensive hemorrhage in all lung lobes. Two females were sacrificed one day after dosing and found to be normal.

Seven Days After Dosing

Two females were sacrificed seven days after dosing and found to be normal. No treated females survived past seven days after treatment due to deaths and sacrifices.

All control females were necropsied at specified time intervals and found to be normal.

MALES

Day of Dosing

On the day of dosing, three treated males were found dead. Two of these males had extensive hemorrhage in all lung lobes. and one had extensive hemorrhage in all lung lobes. the presence of a pale yellow mass. and clear fluid being easily expressed from the lungs. Two males were sacrificed on the day of dosing and found to have red spots in all lung lobes. Finally, one accidental death occurred with necropsy showing extensive hemorrhage in all lung lobes, trachea filled with clear fluid, and clear fluid being easily expressed from the lungs.

One Day After Dosing

One male was found dead and found to have extensive hemorrhage in all lung lobes and the chest cavity filled with bloody fluid. Two males were sacrificed and found to be normal.

7,14, and 21 Days After Dosing

Two treated males were sacrificed and found to be normal on each of these treatment days.

All control males were necropsied at specified time intervals and found to be normal except for animal #2721 which had small testes.

UNCERTAINTIES REGARDING SACRIFICES

Tables 1 and 2 / Male and Female Body Weight Data

It is unclear why female animals 2743, 2744, 2745, 2765, and 2768 do not have body weight data on 7, 14, 21, 7, and 7 days after treatment respectively. It is also unclear why male animals 2713, 2714, 2715, 2733, 2734, 2736, 2738, 2740, and 2741 do not have body weight data on 7, 14, 21, 7, 7, 14, 14, 21, and 21 days after treatment respectively. Although the footnotes state no data is available because the animal was sacrificed for another assay, this is not consistent with those animals such as 2757 and 2758 which had body weights listed on the day of dosing even though they were sacrificed on the day of dosing.

It is unclear why animal weights were recorded in some instances on the day of sacrifice and in other instances not recorded on the day of sacrifice.

III. DISCUSSION:

A. The infectivity/clearance of strain EG2424 is unclear due to the high incidence of mortality and apparent inconsistencies in the data with regards to the presence of EG2424 in lung tissue. The lung excision immediately after dosing is reported as showing no bacteria present. For the male treatment group, day 1 and 7 after treatment show no bacteria present in the lung, day 14 shows bacteria present, and day 21 shows no bacteria present. For the female treatment group, day 1 shows no bacteria and day 7 shows bacteria present.

B. The statement that "the cause of death may be related to congealing of the material and restriction of air." is supported by the rapid onset of deaths and the observations of lung tissue during necropsy.

C. The recovery method that was used to select for strain EG2424 from tissue homogenates is unclear.

D. It is unclear how the effects of heating on the infectivity of the test microbe was investigated and how generated data would be applicable towards understanding the potential infectivity of the test organism. Does 56 degrees C kill spores of the test organism or was the purpose of heating to compare the effects of a spore vs. a spore/vegetative cell preparation? What and how many tissues were tested? How were the tissues exposed to EG2424? How long were the samples heated? Were there controls?

E. It is unclear why animal weights were recorded in some instances on the day of sacrifice and in other instances not recorded on the day of sacrifice.

007368

Reviewed by: Michael Mendelsohn, Microbiologist
PM Team 17, IRB, RD (H7505C) Michael Mendelsohn
Secondary Reviewer: Roy D. Sjoblad, Ph.D., Microbiologist
SACB, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute intravenous toxicity/pathogenicity study in the rat.

MRID NO.: 409511-05 Caswell No.: 66G

TEST MATERIAL: Foil OF Insecticide Basic Formulation Containing 3.97 x 10^8 CFU B.t. subsp. kurstaki strain EG2424/ml

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7138.224

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute intravenous toxicity/pathogenicity study of Foil OF Insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: November 23, 1988

CONCLUSIONS: The test material was not toxic or pathogenic to rats when 0.2 ml of a 3.97 x 10^8 CFU B. t. subsp. kurtsaki strain EG2424/ml preparation was administered via the intravenous route. Although the spleen and lungs of both male and female rats and the liver of a male rat contained strain EG2424 at study termination, strain EG2424 was slowly cleared during the study.

CLASSIFICATION: Unacceptable. Section A, D, and F of the discussion need resolution before this study can be considered acceptable.

I. STUDY DESIGN: A. Test Material: Foil OF Insecticide Basic Formulation assigned MBA chemical No. T07138: received June 8, 1988: containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml (per Ecogen letters dated 4/18/89 and 4/24/89 from Dave Olson to Mike Mendelsohn) diluted to 3.97 x 10^{8} CFU strain EG2424/ml.

B. Test Animals: young adult (approximately 6-7 weeks old at dosing) male and female Sprague Dawley rats; from Harlan Sprague Dawley, Inc., Frederick, MD; Weight: 195-234 g (males) and 117-138 g (females) on day of dosing.

C. Methods: Fifteen male and fifteen female rats each were dosed with 0.2 ml of a 3.97 x 10^{8} CFU strain EG2424/ml preparation of the test material via intravenous dosing (7.94 x 10^{7} CFU strain EG2424/animal). An untreated control group consisted of 15 male and 15 female rats. One hour after dosing, blood samples were plated for the presence of the microbe and infectivity of the test microbe was determined in 2 males and 2 females. Animals were observed for clinical signs of toxicity/disease at 2 and 5 hours after dosing, and then once per day for the 20 days post-treatment or until interim sacrifice. Individual animal body weights were determined at the time of dosing and the weekly during the study. Two male and two female rats from the dosed group and one female and one male from the untreated group were sacrificed 1, 7, 14, and 21 days after dosing for evaluating infectivity. Blood, kidneys, brain, liver, lungs, spleen and, mesenteric lymph nodes from these animals were analyzed for the presence of the test bacterium. All animals survived to study termination (i.e., 21 days after dosing) and were necropsied at this point. In addition, the effects of heating on the infectivity of the test microbe was investigated by heating samples of tissue (unspecified except for lung) and feces exposed to the test microbe to 56 degrees C and comparing plate counts of heated verses non-heated tissue.

II. RESULTS:

A. Body Weights: Body weight gains did not appear to be affected by test article treatment. Mean body weight values were comparable for control and treated animals for each time point.

B. Clinical Signs: No treatment related signs of toxicity were observed at any point during the study.

C. Infectivity Determinations: Significant amounts of microbes were found in the kidney (116 CFUs/g male tissue, 418 CFUs/g female tissue), spleen (1664 CFUs/ g male tissue, 3158 CFUs/g female tissue), lungs (To Numerous to Count/ g male tissue, To Numerous to Count/g female tissue) and liver (156 CFUs/g male tissue, To Numerous to Count/g female tissue) of the treatment groups within 24 hours of injection; small amounts were found in the blood (36 CFUs/ g blood for males and 64 CFUs/ g blood for females). In the female treatment group, EG2424 was found present in brain tissue at 26 and 39 CFUs/g tissue at day 1. After day 1 no EG2424 was found in brain tissue. Within 21 days post treatment, the numbers of colonies were greatly reduced [spleen (68 CFUs/g male tissue, 366 CFUs/g female tissue), lungs (201 CFUs/g male tissue, 217 CFUs/g female tissue), liver (29 CFUs/g female tissue)] or totally cleared from all tissues.

Tissues from control rats were free of colonies at all time points. At the day of dosing, it was determined that low levels (unspecified) of the microbe was present in the blood.

D. Necropsy: Treatment-related lesions were limited to a discoloration of the lungs observed in one treatment group male sacrificed and necropsied on study day 21.

III. DISCUSSION:

A. The recovery method that was used to select for strain EG2424 from tissue homogenates and blood is unclear. This must be specified.

B. It is unclear how the effects of heating on the infectivity of the test microbe was investigated and how generated data would be applicable towards understanding the potential infectivity of the test organism. Does 56 degrees C kill spores of the test organism or was the purpose of heating to compare the effects of a spore vs. a spore/vegetative cell preparation? What and how many tissues were tested? How were the tissues exposed to EG2424? How long were the samples heated? Were there controls?

C. The methods used to estimate the units of bacteria in the test sample and date of analysis should accompany the study report. Where possible, the estimate of bacterial units should be done prior to dosing of the test animals.

D. Levels and presence of the test bacterium in the blood was not reported at the one hour sampling. This should be reported.

E. Clearance of strain EG2424 was shown along with a lack of toxicity and pathogenicity.

F. It is unclear why body weights were not determined for animals 2653-2655, 2671-2676, 2683-2685, and 2701-2706 on the date of necropsy. This should be explained.

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DATA EVALUATION REPORT

STUDY TYPE: Primary eye irritation in rabbits

MRID NO.: 409511-06 Caswell No.: 66G

TEST MATERIAL: Foil OF Insecticide Basic Formulation Containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7138.230

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Primary Eye Irritation Study of Foil OF Insecticide in Albino Rabbits

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: November 21, 1988

CONCLUSIONS: Although a 1/30 dilution of the basic formulation was found to be in Toxicity Category III, the potential for the end-use product to cause eye irritation cannot be determined based on the test substance used.

CLASSIFICATION: Unacceptable.

I. STUDY DESIGN: A. Test Material: Foil OF Insecticide Basic Formulation assigned MBA chemical No. T07138 (lot number 178-31); received June 8, 1988; containing 2.38 x 10^{10} CFU B.t. subsp. kurstaki strain EG2424/ml 'per Ecogen letters dated 4/18/89 and 4/24/89 from Dave Olson to Mike Mendelsohn).

B. Test Animals: young adult (approximately 10 weeks old at dosing) male New Zealand albino rabbits, from Hazelton Research Products. Denver, PA; Weight: 1.9-2.0 kg on day of dosing.

C. Methods: The dosing material was a preparation of the test article which had been diluted in saline (1 ml of the test article in 29 ml saline) to give a bacterial concentration of 7.9 x 10^{8} CFU/ml. One-tenth ml of the dosing material was instilled into the lower eyelid of each of six test animals. Upper and lower eyelids of treated eyes then were held together for 2-3 seconds. The untreated right eye of each animal served as the representative control for each animal. All eyes were examined for defects prior to dosing, and only the healthy eyes were used in the study. Ocular lesions were recorded and scored (Draize method) at 1 hour and at 1,2,3, and 4 days after dosing. Corneal opacity was recorded via the fluorescein dye method. Individual animal body weights were recorded at animal selection and at the time of dosing. Animals were observed daily for mortality and for clinical signs of toxicity.

II. RESULTS: The test material caused slight erythema in three animals (score=1), moderate erythema in three animals (score=2), moderate discharge in four animals (score=1), and a score of 1 for the iris one hour after application of the test material. Twenty-four hours after administration, one animal received a score of 1 for discharge and three animals received a score for redness. No ocular lesions were observed 48, 72, 96, and 168 hours after administration.

III. DISCUSSION:

A. The dosing material should have consisted of the undiluted end-use product.

B. The 1/30 dilution of the end-use product tested produced irritation 1 and 24 hours after application and falls in Toxicity Category III. This dilution does not support the end-use product.