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

MAY 16 1989

007168

MAY 16 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: SACB consideration of Product analysis/residue chemistry and mammalian toxicology data submitted by Ecogen, Inc. to support the registration of Condor® OF Insecticide for Forestry use. (ID No. 55638-T; Record No. 234,886, MRID Nos. 408988-01 through 408988-06, HED Project No. 9-0420 and 9-0420A; Caswell No. 66G).

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

FROM: Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Roy D. Sjoblad 5/11/89

and

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

W. J. Hazel 5/11/89

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

Reto Engler 5/12/89

Action requested: Ecogen, Inc. wishes to register Condor™ OF insecticide for forest use in controlling certain insect pests (gypsy moth and spruce budworm). The active bacterial ingredient of the insecticide is a transconjugant strain of Bacillus thuringiensis var. kurstaki (strain EG2348). Application of the insecticide is by either conventional ground equipment, or by aerial equipment. Application rates are designated as up to 2 pints/10 gallons (ground equipment) or up to 2.5 pints/acre (aerial application). The proposed product label requires that applicators, mixers, and loaders are to wear goggles and a particle mask. In 1988, Ecogen, Inc. received an EUP to conduct an experiment with Condor™ OF insecticide on 895 total acres of forest land. Deficiencies requiring satisfactory resolution prior to granting of a Section 3 registration were identified by W. Hazel (5/27/88 Memorandum). Transconjugant strains of B. thuringiensis, including EG 2348, are subject to the data requirements of the Bt Registration Standard which was issued on 12/88. Thus, certain requirements may have changed from the time of granting of the EUP to issuance of the Registration Standard.

Recommendations: The information/data submitted on the product analysis and residue chemistry are not adequate to allow SACB to recommend registration of Condor™ OF insecticide. If certain information (i.e., whether an MP or equivalent is to be sold; see Product Identity section of this Memorandum for specific information requested) is supplied then SACB may be able to recommend that a conditional registration be granted. Deficiencies that remain to be resolved largely reflect specific data/information that are specified as requirements in the Bt Registration Standard, issued 12/88.

The mammalian toxicity/pathogenicity data submitted are not adequate to allow SACB to recommend that a registration for Condor™ OF insecticide be granted. The acute oral infectivity/pathogenicity study is inadequate to show that the active bacterial ingredient was delivered to test animals, and thus a pattern of clearance cannot be determined. The acute pulmonary study is inadequate to show that the active bacterial cannot infect (i.e., replicate in) the lungs. The acute intravenous study is adequate to support the registration of the active ingredient. The acute oral, acute dermal, acute eye toxicity/irritation studies are inadequate to support registration of the end use product because a diluted preparation (i.e., 1:250 dilution) of the end use product was used as the dosing material.

In view of the above stated inadequacies, SACB would consider recommending that a conditional registration of Condor™ OF insecticide be granted for the following reasons: Food-uses are not proposed and that the label adequately reflects worker/applicator protection via pulmonary and ocular coverings, and via appropriate washing procedures. To further support recommendation of a conditional registration, the Registrant, as requested, has submitted a report on observations relating to any adverse human health effects resulting from the EUP program with Condor OF insecticide conducted in 1988. The results from the report indicate, qualitatively, that significant exposure of mixers, pilots, and "flagmen" to Condor™ OF did occur, and that observations indicated no adverse reactions from the exposure under conditions of the EUP.

In order for SACB to recommend unconditional registration of Condor Of insecticide, all inadequacies of the currently submitted mammalian toxicity/pathogenicity studies must be resolved. In addition, as specified in the Bt Registration Standard, issued 12/88, an appropriate intraperitoneal injection study is to be done.

Product Identity

To avoid repetition, only unresolved issues, unsatisfied requirements, and data not reviewed in the 5/27/88 memorandum will be discussed here.

151A-10. Product identity and disclosure of ingredients. The composition of the end-use product (EP) has been altered somewhat (see 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, and identity of insecticidal toxins produced have been adequately defined. The following additional data are required but SACB recommends allowing conditional registration pending their satisfactory submission:

- 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 the plasmid profiles are acceptable, a statement should be made as to which plasmids encode a toxin.

- Each crystalline endotoxin (inclusion) must be described in terms of morphology.

- Although representatives of the insect orders Coleoptera, Hymenoptera, and Lepidoptera have been tested for their sensitivity to EG2348, 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.

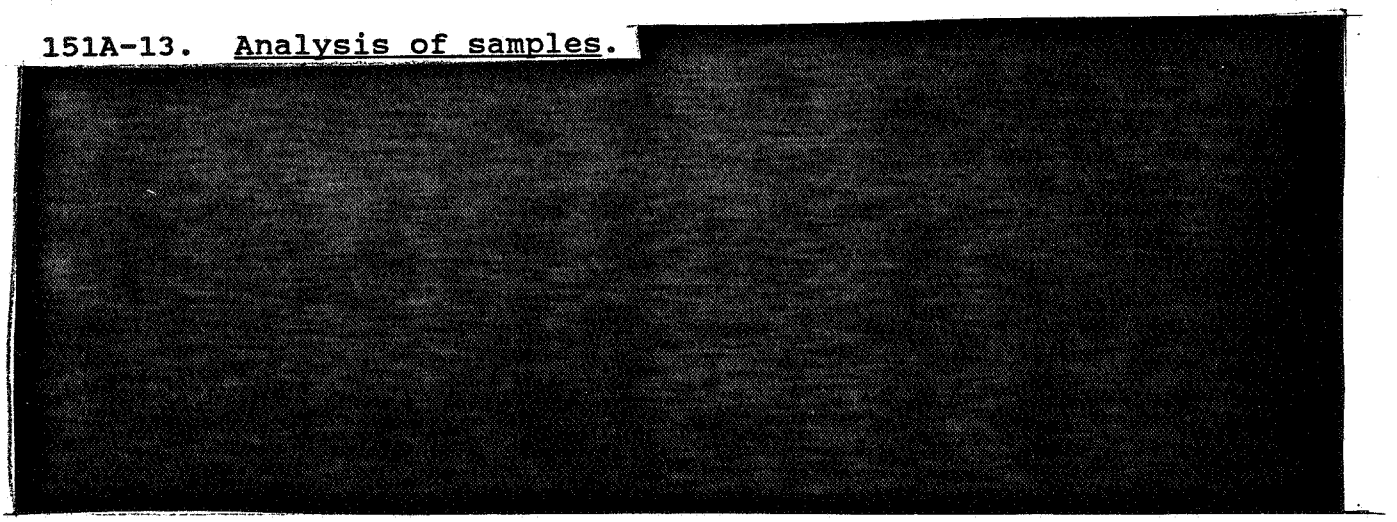
The following must be satisfied prior to conditional registration:

- 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 5/27/88 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 5/27/88 review have been resolved. No deficiencies remain.

151A-13. Analysis of samples.



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 Condor OF and the MP must be analyzed such that insect order bioactivity can be determined eg., %(w:w) lepidopteran-active toxin(s) and/or %(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 P2-type toxins) of Condor 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. If endotoxins active against more than one insect order are present in Condor 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. Similarly, no significant loss of biological activity occurred within 48 hr at 20 C at pH 5, 7, and 9.

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 represents a conditional registration requirement:

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

Residue Chemistry

Proposed use directions. The 7.5% OF is proposed for use at 1.5-2.5 pints product/A to control gypsy moth and spruce budworm larvae in forests and on shade trees and shrubs. Aerial or ground applications are to be made when larvae are young and actively feeding, first/second instars of gypsy moth and third/fourth instars of spruce budworm. A sticker may be used. The following amendments should be made to the OF label:

- The number of applications to be made per season and the interval between treatments must be specified.

- The Bt strain designation, EG2348, should be listed on the 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.

MAMMALIAN TOXICOLOGY

Introduction.

Ecogen, Inc. has submitted the following mammalian toxicology studies, and believes that, "...since the administered doses follow the guidelines for both active ingredient and end use product, hazards due to both the active ingredient and the formulation have been addressed." Mammalian toxicology data were included to address the requirements for a tolerance exemption, and believes it is important to address "...any concerns about inadvertant residues resulting from label uses or the food use of any plants or plant parts normally associated with forest habitats...". At the request of SACB, Registration Division has asked for, and received from the Registrant, a documentation of worker/applicator exposure to Condor and any adverse health effects observed upon conduct of the 1988 EUP program (5/10/89 letter from D.L. Olson of Ecogen, Inc. to P. Hutton).

The submitted studies and exposure documentation report 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 (1:250 dilution) was not toxic to or pathogenic for rats, however, no colony-forming units of the active bacterial ingredient were isolated from any tissue/organ/body fluid material at any time during the study.

§152A-11. Acute dermal toxicity.

An unacceptable acute dermal toxicity study was submitted. A diluted preparation (0.5 ml test article/124.5 ml saline) was not toxic to rabbits when applied dermally for 24 hours. The study is unacceptable because a diluted preparation of test material was used, and signs of skin irritation were not recorded.

§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, and were not adequate to rule out the possibility of significant bacterial replication in test animal lungs. Under the conditions of the study, the test material did not cause toxicity or pathogenicity in rats.

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

An acceptable acute intravenous toxicity/pathogenicity study in rats was submitted. The data were adequate to show that the active bacterial ingredient was not toxic to or pathogenic for test animals after intravenous injection. The data showed that the active bacterial ingredient is slowly, but steadily cleared from the test animals.

§152A-14. Primary eye irritation.

The primary eye irritation study submitted was unacceptable because it was not indicated clearly whether diluted or undiluted test material 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, caused only slight erythema, and slight discharge in 2-3/5 test rabbits only at the 1 hour post-dosing observation time. Since goggles are to be worn by applicators, mixers, and loaders, this study may not have to be repeated.

Other.

The number of units in the test article material should be determined just prior to dosing of test animals.

Documentation of exposure and any observed adverse health effects from conduct of EUP program.

No adverse human health effects were observed in personnel during extensive forest applications of Condor™ OF from the years 1986-1988 on 165 acres in Delaware, 125 acres in New Jersey, and 375 acres in Pennsylvania. Persons exposed to the pesticide included: mixers who pumped the product from 55 gallon drums to mixing tanks; personnel calibrating aircraft (including a helicopter while rotors were running); pilots upon reexposure to previously sprayed swath; grounds people deploying balloons to guide pilot; personnel cleaning the aircraft; and, personnel who reentered test areas to evaluate field persistence of the pesticide.

The cooperator responsible for most of the aerial applications of Condor, and a different cooperator from Pennsylvania have expressed that there have been no findings of adverse reactions to the product in any of the exposed personnel.

Reviewed by: Roy D. Sjoblad, Ph.D. R. D. Sjoblad 5/11/89
Section: Science Support Staff, Science Analysis and Coordination Branch (H-7509C)
Secondary reviewer: Reto Engler, Ph.D. 5/12/89
Chief, SACB (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity/pathogenicity in rat

MRID NUMBER: 408988-02 CASWELL NO.: 66G

TEST MATERIAL: Condor® OF Insecticide

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7095.222

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute oral toxicity/pathogenicity study of Condor OF insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: June 6, 1988

CONCLUSIONS: Toxicity/infectivity/clearance of the bacterium from test animals could not be determined from the data submitted.

Classification: Unacceptable; because data were insufficient to establish a pattern of clearance from test animals. No test bacteria were detected in any sample at any time during the study.

I. STUDY DESIGN: A. Test material: Condor OF Insecticide (lot # 178-23) assigned MBA chemical No. T07095; received April 11, 1988; 2.5×10^{10} CFU B. thuringiensis (B.t.)/ml.

B. Test animals: young adult (approx. 7 weeks old at dosing) male and female CD rat; from Charles River Laboratories, Raleigh, N.C.; Weight: 199-264 g (males) and 150-194 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 the test material diluted to contain 10^8 CFU B.t./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 1 and at 4 hours after dosing, and then once per day for 22 days 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 control group were sacrificed at 1 day, and at 7 days, and at 14 days after dosing. 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.

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 the treated and control groups. No test-substance related lesions were observed in any test animal at the 21-day terminal sacrifice time. No CFU of the active bacterial ingredient were detected on growth media upon plating homogenates of any tissue/organ from any test animal at any interim sacrifice time. Also, no bacteria were detected in the fecal samples from test animals.

III. DISCUSSION:

A. The appropriate dosing material to be used in this acute oral study would have been the undiluted Condor® OF insecticide.

B. Since no bacterial colonies were detected at any time in any test animal, there is no assurance that the methods used were reliable for verification of the absence of the active bacterial ingredient. In addition, it cannot be determined whether the test material contained bacterial spores of the active ingredient which could germinate on appropriate growth medium.

C. At the very least, a properly conducted acute oral study would have allowed detection of the active bacterial ingredient in the feces of test animals. It appears that fecal sampling frequencies were inadequate to establish a pattern of clearance of the test organism from the gastrointestinal tract of test animals.

D. The study should be repeated using an undiluted test material, and in which the number of active bacterial units in the dosing material is determined - preferably at the time of dosing - and fecal sample analyses should be done sufficiently frequently so that a pattern of clearance can be determined.

E. 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 also should be done just prior to dosing of the test animals.

Reviewed by: Roy D. Sjoblad, Ph.D. R.D. Sjoblad 5/11/89
Section: Science Support Staff, Science Analysis and Coordination Branch (H-7509C)
Secondary reviewer: Reto Engler, Ph.D. 5/12/89
Chief, SACB (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute pulmonary toxicity/pathogenicity in rat

MRID NUMBER: 408988-04 CASWELL NO.: 66G

TEST MATERIAL: Condor® OF Insecticide

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7095.225

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute pulmonary toxicity/pathogenicity study of Condor OF insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: June 6, 1988

CONCLUSIONS: The data presented are insufficient to allow for conclusions to be made on the ability of test animals to clear the test bacterium (i.e., on "unusual persistence") after pulmonary exposure, and also on the ability of the test bacterium to replicate in animal lung tissue. Under the conditions of the study, the test material did not cause disease or overt signs of toxicity.

Classification: Unacceptable.

I. STUDY DESIGN: A. Test material: Condor OF Insecticide (lot # 178-23); assigned MBA chemical No. T07095; received April 11, 1988; 2.5×10^{10} CFU B. thuringiensis (B.t.)/ml.

B. Test animals: young adult (approx. 7 weeks old at dosing) male and female CD rat; from Charles River Laboratories, Raleigh, N.C.; Weight: 212-264 g (males) and 161-203 g (females) on day of dosing.

C. Methods: Fifteen male and fifteen female rats each were dosed intratracheally with 0.04 ml of the test material diluted to contain 2×10^9 CFU B.t./ml. An untreated control group consisted of 15 male and 15 female rats. Animals were observed for clinical signs of toxicity/disease at 1 and at 4 hours after dosing, and then once per day for 21 days 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 control group each were sacrificed at 1 day, 7 days, 14 days and 21 days after dosing. The blood, kidneys, brain, liver, spleen, lungs and mesenteric lymph nodes from these animals were analyzed for the presence of the test bacterium. Lungs (from two treated male and two treated female animals and from one untreated male and one untreated female) also were analyzed within one hour after dosing. All animals surviving to study termination (i.e., to 21 days after dosing) were necropsied.

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 the treated and control groups. No clinical signs of toxicity were observed in treated animals. At terminal sacrifice, 3/5 treated males and 1/5 treated females showed areas of grey consolidation in the lungs. Viable active ingredient bacteria were isolated from the lungs on the day of dosing: "...a 1:30 dilution of the lungs yielded an average of 29 colonies per plate for males and 32 for females." No bacteria were detected in the lungs of one male animal at day 1. The data indicated that the active bacterial ingredient might have replicated in the lungs of treated animals since the values at the day 7 analyses were higher than the day 1 analyses. A general very slow pattern of clearance from the lungs was observed upon analyzing lungs at 14 days and at 21 days after dosing. Viable test bacteria were not detected in any other tissue/organ of treated animals, except for the sole detection of 2.2×10^3 CFU/gram of liver tissue from one female at day 7.

III. DISCUSSION:

A. The number of CFU/g lungs from the 1 hour post-dosing time should be presented. The data presented indicate that although the active bacterial ingredient can replicate in animal lung tissue, it is not able to cross the lung barrier and thus is not detected in other organs, or even in the blood of the test animals. The test organism is only very slowly cleared from the lungs of the test animals. On the other hand, the study data may reflect problems in recovery of bacteria after delivery of a sufficiently high dose of the test bacterium in a very small volume (0.04 ml) of delivery material.

B. 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 also should be done just prior to dosing of the test animals.

Reviewed by: Roy D. Sjoblad, Ph.D. R.D. Sjoblad 5/11/89
Section: Science Support Staff, Science Analysis and Coordination Branch (H-7509C)
Secondary reviewer: Reto Engler, Ph.D. 5/12/89
Chief, SACB (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute intravenous toxicity/pathogenicity in rat

MRID NUMBER: 408988-05 CASWELL NO.: 66G

TEST MATERIAL: Condor® OF Insecticide

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7095.224

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute intravenous toxicity/pathogenicity study of Condor OF insecticide in rats.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: June 6, 1988

CONCLUSIONS: The test material was not toxic to, nor caused disease symptoms in rats after intravenous injection of 10^7 CFU/animal. The data indicated that the test bacterium was slowly but steadily cleared from the test animals.

Classification: Acceptable.

I. STUDY DESIGN: A. Test material: Condor OF Insecticide (lot # 178-23; assigned MBA chemical No. T07095; received April 11, 1988; 2.5×10^{10} CFU B. thuringiensis (B.t.)/ml.

B. Test animals: young adult (approx. 7 weeks old at dosing) male and female CD rat; from Charles River Laboratories, Raleigh, N.C.; Weight: 240-295 g (males) and 185-215 g (females) on day of dosing.

C. Methods: Fifteen male and fifteen female rats each were dosed via intravenous injection with 0.1 ml of the test material diluted to contain 2×10^8 CFU B.t./ml. An untreated control group consisted of 15 male and 15 female rats. Animals were observed for clinical signs of toxicity/disease at 1 and at 4 hours after dosing, and then once per day for 21 days 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 control group each were sacrificed at 1 day, 7 days, 14 days and 21 days after dosing. The blood, kidneys, brain, liver, spleen, lungs and mesenteric lymph nodes from these animals were analyzed for the presence of the test bacterium. Blood (from the abdominal aorta of two treated male and two treated female animals and from one untreated male and one untreated female) also was analyzed within one hour after dosing. All animals surviving to study termination (i.e., to 21 days after dosing) were necropsied.

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 the treated and control groups. No clinical signs of toxicity were observed in treated animals. No lesions were observed in any dosed animals at terminal sacrifice. On the day of dosing, 10 CFU/ml of blood were detected in treated animals. At 24 hours after dosing, from 30-150 CFU/ml of blood were detected in 3/4 test animals. None were detected in the blood of the fourth test animal at 24 hours, and no CFU were found in the blood from any test animal at subsequent sampling times. At 24 hours, lungs from test animals contained from $1.5\text{-}2.8\text{x}10^3$ CFU/g which decreased to $0\text{-}3\text{x}10^2$ at 21 days. At 24 hours, livers from test animals contained from 60-1,260 CFU/g which decreased to 0-120 CFU/g at 21 days. Kidneys from 3/4 animals contained from 30-270 CFU/g at 24 hours, but none at days 14 and 21. Lymph nodes were free of B.t. CFU at days 7, 14, and 21. No CFU were isolated from brain tissue at any sampling time. At 24 hours, spleens contained from 60-630 CFU/g, which decreased to 0 CFU/g in two test animals, and to 30 and 60 CFU/g in the other two animals at 21 days.

III. DISCUSSION:

A. The data are consistent with a slow but steady processing of the injected bacterium by the immune system organs of the test animals.

B. 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 also should be done just prior to dosing of the test animals.

Reviewed by: Roy D. Sjoblad, Ph.D. R.D. Sjoblad 5/11/89
Section: Science Support Staff, Science Analysis and Coordination Branch (H-7509C)
Secondary reviewer: Reto Engler, Ph.D. R 5/12/89
Chief, SACB (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity in rabbit

MRID NUMBER: 408988-03 CASWELL NO.: 66G

TEST MATERIAL: Condor® OF Insecticide

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7095.232

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Acute dermal toxicity study of Condor OF insecticide in rabbits.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: September 6, 1988

CONCLUSIONS: Diluted test material (0.5 ml in 124.5 ml saline) was not toxic when applied at 1 ml to the skin of rabbits.

Classification: Unacceptable; because diluted test material was used, and signs of skin irritation were not recorded.

I. STUDY DESIGN: A. Test material: Condor OF Insecticide (lot #178-23) assigned MBA chemical No. T07095; received April 11, 1988; 2.5×10^{10} B. thuringiensis (B.t.) spores/ml.

B. Test animals: young adult (approx. 9 weeks old at dosing) male and female New Zealand albino rabbits, from Hazleton 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 test animals. The dosing material was a preparation of the test article which had been diluted in saline (0.5 ml test article in 124.5 ml saline) to give a bacterial concentration of 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 two times on the day of dosing, and then at least 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: One female animal showed signs of diarrhea and lethargy on day 9 after dosing, and died on day 11. No lesions were observed upon necropsy in this or in any other test animal. All surviving animals gained weight during the study.

III. DISCUSSION:

A. The dosing material should have consisted of undiluted test article material (with weight of dosing material applied to each animal recorded), and observations of the skin for signs of irritation also should have been made and recorded.

B. The one instance of mortality was probably not related to toxic properties of the test substance, however, the data did not allow for a determination of the cause of death.

Reviewed by: Roy D. Sjoblad, Ph.D. R.D. Sjoblad 5/11/89
Section: Science Support Staff, Science Analysis and Coordination Branch (H-7509C)
Secondary reviewer: Reto Engler, Ph.D. R 5/12/89
Chief, SACB (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary eye irritation in rabbit

MRID NUMBER: 408988-06 CASWELL NO.: 66G

TEST MATERIAL: Condor® OF Insecticide

SYNONYMS: Bacillus thuringiensis transconjugant

STUDY NUMBER: G-7095.230

SPONSOR: Ecogen, Inc.

TESTING FACILITY: Microbiological Associates Inc.

TITLE OF REPORT: Primary eye irritation study of Condor OF insecticide in rabbits.

AUTHORS: Raymond M. David, Ph.D.

REPORT ISSUED: September 6, 1988

CONCLUSIONS: The potential for the end-use product to cause eye irritation cannot be determined, because it was not indicated clearly that undiluted test article was used as the test substance.

Classification: Unacceptable; however, study need not be repeated because label precautionary statement requires that applicators, mixers, and loaders were protective eye coverings (i.e., goggles).

I. STUDY DESIGN: A. Test material: Condor OF Insecticide (lot #178-23) assigned MBA chemical No. T07095; received April 11, 1988; 2.5×10^{10} B. thuringiensis (B.t.) spores/ml.

B. Test animals: young adult (approx. 9 weeks old at dosing) male New Zealand albino rabbits, from Hazleton Research Products, Denver, PA; Weight: 2.0-2.4 kg on day of dosing.

C. Methods: One-tenth ml of the test material was instilled into the lower left eyelid of each of six test animals. The dosing material was a preparation of the test article which had been diluted in saline (0.5 ml test article in 124.5 ml saline) to give a bacterial concentration of 10^8 CFU/ml. However, in a separate section of the study report, it was stated that the undiluted test article was used. 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 respective control for each animal. All eyes were examined for defects prior to dosing, and only 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 at 1 day after dosing via the fluorescein dye method. Individual animal body weights were recorded at the time of dosing and at 7 days after dosing. Animals were observed daily for mortaity and for clinical signs of toxicity.

II. RESULTS: The test material caused slight erythema (score=1) in treated eyes of 2/5 test animals, and slight discharge (score=1) in 3/5 animals at the 1 hour post-treatment observation time. No other signs of eye irritation were observed at the 1 hour or at any subsequent observation times.

III. DISCUSSION:

A. The dosing material should have consisted of undiluted test article material. At two places in the report it was indicated that diluted test article was used, and in one place it was indicated that undiluted material was used. SACB is assuming that the diluted form of the test material was used. The Registrant clarified.

B. The potential for the end-use product to act as an eye irritant cannot be detrmined because it was not clear whether undilited test material was used. Therefore, the study is unacceptable. However, the study need not be repeated because the proposed label indicates that eye coverings (goggles) are to be worn by applicators, mixers and loaders.