9/19/89

Summary of studies submitted:

152A-10. Acute oral toxicity/pathogenicity - rat (MRID No. 413086-1) (Amended study to upgrade MRID No. 409511-02)

Classification: Acceptable to upgrade earlier study and to support registration of the technical grade of the active ingredient.

The original study can be upgraded to Acceptable since the Registrant has provided information on the techniques used to enumerate bacteria from test animal tissues and feces, and missing body weight data were provided.

152A-10. Acute oral toxicity study - mouse (MRID No. 413086-04)

Classification: Acceptable to support registration of the end use product.

Foil OF was not lethal for or toxic to mice when dosed with the test material at 5g/kg (a concentration equivalent to a dose level of B. thuringiensis var. kurstaki strain EG 2424 at $3x10^9$ CFU/animal.

152A-11. Acute dermal toxicity - rabbit (MRID No. 413086-06)

Classification: Acceptable Tox Category: II

Foil OF caused moderate to severe erythema (Maximum PIS = 4.0 for males at days 4-6 after dosing; and 3.6 for females at 4 days). Severe erythema was observed for up to 12 days after dosing in some animals.

152A-12 Acute pulmonary (intratracheal) toxicity/pathogenicity - mouse (MRID No. 413086-03).

Classification: Acceptable

Foil OF was not lethal for or toxic to mice when diluted to give a dose level of $1.5x10^6$ CFU/animal. Autoclaved and non-autoclaved Foil OF containing B.t.k. at $4.7x10^7$ CFU/animal were lethally toxic for all mice dosed. Non autoclaved Foil Technical Powder killed 3/18 dosed mice, while autoclaved Foil Technical powder was not lethal for or toxic to mice. The active bacterial ingredient was slowly but steadily cleared from mice that survived dosing with Foil OF or Foil Technical Powder.

152A-13 Acute intravenous toxicity/pathogenicity - rat (MRID No. 413086-02; Amended study to upgrade MRID No. 409511-05).

Classification: Acceptable to upgrade earlier study.

The method used to recover bacteria from test animals was described; missing animal body weight data were supplied;

and, the 1 hour post dosing numbers of bacteria in the blood were provided.

--- Acute intraperitoneal injection study - mouse (MRID No. 413086-07)

Classification: Acceptable.

Foil Technical Powder was not lethal for or toxic to mice when injected at a concentration equivalent to dosing each animal with 1.7×10^8 B.t.k. strain EG2424 viable or killed units/animal (the highest dose tested).

--- Acute pulmonary toxicity study - mouse (MRID No. 413086-05)

Classification: Acceptable to support registration of the formulated oil flowable product.

The oil flowable product was lethally toxic for all mice when dosed at 5 mg/animal; and to 2/5 males and 1/5 females at 0.5 mg/animal. The oil flowable vehicle was not lethal for or toxic to mice when dosed at 0.05 or 0.005 mg/animal. The only gross lesion noted in animals that died were uniformly red lungs.

007788

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist RDS
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C) RE

DATA EVALUATION REPORT

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

MRID NO.: 413086-01 (Current study) Caswell No: 66G
409511-02 (Original submitted study)

TEST MATERIAL: Foil OF Insecticide Basic Formulation containing 2.38×10^{10} CFU/ml of B.t. var. kurstaki strain EG2424.

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.

AUTHOR: Raymond M. David, Ph.D.

REPORT ISSUED: November 23, 1988 (Original)
September 19, 1989 (Amended)

CONCLUSIONS: The relevant questions raised by SACB in the 7/27/89 Memorandum have been adequately addressed.

Classification: Acceptable to support registration of the technical grade of the active ingredient.

See Memorandum of July 27, 1989 from W. Hazel and R. Sjoblad to P. Hutton/M. Mendelsohn for detailed review of the study.

The study was classified as Unacceptable because the recovery method used to select for strain EG2424 from animal tissues, blood and feces was not clear, and because body weight data were not provided for animals 2602, 2615-2618, and 2641-2644. In addition, since a diluted preparation of the test material was used, the study was not adequate to support registration of the end-use product. The following was offered in the amended report to address these issues:

The test microorganism was enumerated from animal samples by plating tissue/blood/fecal samples/homogenates (or appropriate dilutions of the samples/homogenates) onto antibiotic-supplemented nutrient agar plates. Strain EG2424 was identified by colony morphology. Recovery studies with samples "spiked" with EG2424 gave an average recovery value of 86%. Body weight values were provided for those animals mentioned above. Although not presented in the original report, the individual animal body weights were included in the summary values; and hence, the original conclusion that the test material as administered had no significant effect on mean body weight gains still is valid.

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Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist RDS
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C) RE

DATA EVALUATION REPORT

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

MRID NO.: 413086-02 (Current study) Caswell No: 66G
409511-05 (Original submitted study)

TEST MATERIAL: Foil OF Insecticide Basic Formulation containing 3.97×10^8 CFU/ml of B.t. var. kurstaki strain EG2424.

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.

AUTHOR: Raymond M. David, Ph.D.

REPORT ISSUED: November 28, 1988 (Original)
September 19, 1989 (Amended)

CONCLUSIONS: The relevant questions raised by SACB in the 7/27/89 Memorandum have been adequately addressed.

Classification: The study can be upgraded to Acceptable.

See Memorandum of July 27, 1989 from W. Hazel and R. Sjoblad to P. Hutton/M. Mendelsohn for detailed review of the study.

The study was classified as Unacceptable because the recovery method used to select for strain EG2424 from animal tissues, blood and feces was not clear, and because body weight data were not provided for animals 2653-2655, 2671-2676, 2683-2685, and 2701-2706. In addition, numbers of bacteria in blood at the 1 hour sampling time were not reported. These items were addressed in the amended report as follows:

The test microorganism was enumerated from animal samples by plating tissue/blood samples/homogenates (or appropriate dilutions of the samples/homogenates) onto nutrient agar plates supplemented with ampicillin (50 mg/ml) and Polymixin B sulfate (10^5 Units/L). Strain EG2424 was identified by colony morphology. Recovery studies with lungs "spiked" with EG2424 gave an average recovery value of 124%. Body weight values were provided for those animals mentioned above. Although not presented in the original report, the individual animal body weights were included in the summary values; and hence, the original conclusion that the test material as administered had no significant effect on mean body weight gains still is valid. At one hour after dosing, an average of 30 CFU and 12 CFU were detected per ml of blood respectively from females and from males.

007788

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity study in the rabbit.

MRID NO.: 413086-06 Caswell No: 66G

TEST MATERIAL: Foil OF, a formulation containing B.t. var. kurstaki strain EG2424.

STUDY NUMBER: Study No. 8 of Project No. L08239

SPONSOR: Ecogen, Inc.

TESTING FACILITY: IIT Research Institute

TITLE OF REPORT: EPA Subdivision M acute dermal toxicity testing of Foil® Oil Flowable biopesticide.

AUTHOR: Robert L. Sherwood, Ph.D.

REPORT ISSUED: November 21, 1989

CONCLUSIONS: The test material caused moderate to severe erythema with a mean combined maximum PIS of 3.8 at day 4 after dosing. Signs of severe erythema were observed in some animals at 11-12 days after dosing; with the most severe reaction at 4-7 days.

Classification: Acceptable.

1. STUDY DESIGN : A. Test Material: Foil Oil Flowable (Lot #179-36 023A0401) containing B. thuringiensis var. kurstaki at approximately 2.5×10^{10} CFU/gram. B. Test animals: New Zealand albino rabbits (from Johnson Rabbit Ranch; Wilkinson, IN); Mean weight at dosing: 2.66 kg (males) and 2.53 kg (females).

C. Methods: Five male and five female rabbits each were dosed with undiluted test material at 2g/animal; via application to the shaved right flank. Test sites were covered with adhesive dressing. Adhesive dressing also was applied to the shaved but untreated left flank of each animal. The midsection of each animal was wrapped with sterile lint-free cloth, and then with an elastic adhesive bandage. At 24 hours after application of test material, all wrappings/adhesive materials were removed and skin was wiped clean with gauze moistened with 0.9% saline. Each animal was observed daily for clinical signs of toxicity, morbidity, and mortality. Body weights were measured at the time of dosing and weekly thereafter. Each animal was scored for signs of skin irritation (erythema, eschar formation, edema; according to Draize, 1944) at the time of test article removal and then daily for 14 days.

D. Results: None of the test animals died and no clinical signs of toxicity were noted. Mean body weights for females and males increased during the study. Slight edema was observed in one

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female rabbit at 3 days after dosing. Moderate to severe erythema was observed in all male and in all female rabbits. By day 13, the treated skin of all animals appeared normal. The maximum mean dermal irritation score for males was 4.0/8.0, at days 4-6 after dosing. The maximum mean dermal irritation score for females was 3.6/8.0 observed at 4 days after dosing. The maximum mean combined dermal irritation score was 3.8/8.0 at 4 days after test material application.

E. Discussion.

1. In future submissions, the Registrant should submit individual animal body weights and also individual animal irritation scores (for erythema, edema, and other signs).

2. The test material can be categorized as a TOX Category II dermal irritant. Severe erythema was noted in all test animals, with the most severe effects noted at days 4 through 7. Also, there was indication of a delayed irritation response, at least in several animals where the erythema lessened at day 3, and then increased to severe erythema at 4 days. Finally, several animals experienced severe erythema even at 11-12 days after dosing.

007788

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist RDS
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity study in the mouse.

MRID NO.: 413086-04 Caswell No: 66G

TEST MATERIAL: Foil OF, a formulation containing B.t. var. kurstaki strain EG2424.

STUDY NUMBER: Study No. 4A of Project No. L08245

SPONSOR: Ecogen, Inc.

TESTING FACILITY: IIT Research Institute

TITLE OF REPORT: Acute Oral Toxicity Limit Testing of Foil® Oil Flowable Biopesticide.

AUTHOR: Robert L. Sherwood, Ph.D.

REPORT ISSUED: November 27, 1989

CONCLUSIONS: The test material at any dose tested was not toxic to or lethal for mice. The highest dose tested was 5g/kg containing about $3x10^9$ units of active bacterial ingredient.

Classification: Acceptable.

1. STUDY DESIGN : A. Test Material: Foil Oil Flowable (Lot #179-36 023A0401) containing B. thuringiensis var. kurstaki at approximately $2.5x10^{10}$ CFU/gram. B. Test animals: CD1 mouse (from Charles River Laboratories; Portage, MI); Weight at dosing: 23.4-24.3 g (males) and 19.8-20.4 g (females).

C. Methods: Male and female mice were dosed by gavage with test material at 0, 0.1, 0.5, 1.0, or 5 g/kg animal. There were 5 animals/sex/dose group. The test material was suspended in sterile saline (0.9%) and each animal received 0.1 ml/g body weight, except for the highest dose group which received 0.02 ml/g. Each animal was observed daily for clinical signs of toxicity, morbidity, and mortality. Body weights were measured at the time of dosing and at study termination (14 days after dosing).

D. Results: None of the test animals died and no clinical signs of toxicity were noted. The test material had no effect on weight gain in test animals. At the highest dose tested, each animal received about $3x10^9$ units of the active bacterial ingredient.

007788

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute intraperitoneal injection toxicity study in the mouse.

MRID NO.: 413086-07 Caswell No: 66G

TEST MATERIAL: Foil® technical powder containing B.t. var. kurstaki strain EG2424.

STUDY NUMBER: Study No. 7 of Project No. L08239

SPONSOR: Ecogen, Inc.

TESTING FACILITY: IIT Research Institute

TITLE OF REPORT: Acute Intraperitoneal Toxicity/Pathogenicity Testing of Foil® Technical Powder, a Microbial Pesticide.

AUTHOR: Robert L. Sherwood, Ph.D.

REPORT ISSUED: November 20, 1989

CONCLUSIONS: The test material at any dose tested was not toxic to or lethal for mice when injected intraperitoneally in a single dose containing up to 1.7×10^8 units of viable or non-viable bacterial ingredient.

Classification: Acceptable.

1. STUDY DESIGN : A. Test Material: Foil Technical Powder (Lot PP0009) containing B. thuringiensis var. kurstaki at approximately 1.1×10^{11} CFU/gram. B. Test animals: CD1 mouse (from Charles River Laboratories; Portage, MI); Mean weight at dosing: 24.3 g (males) and 22.9 g (females).

C. Methods: Male and female mice were dosed by intraperitoneal injection of a single dose of test material at 1.7×10^6, or 1.7×10^7, or 1.7×10^8 CFU/animal. There were 5 animals/sex/dose group. The test material was suspended in sterile saline (0.9%) and each animal received 0.2 ml. An additional dose group was injected with the 1.7×10^8 CFU dose material which had been autoclaved prior to i.p. injection. The effectiveness of autoclaving in killing the bacterium was determined. Each animal was observed daily for clinical signs of toxicity, morbidity, and mortality. Body weights were measured at the time of dosing.

D. Results: None of the test animals died and no clinical signs of toxicity were observed except for the appearance of "rough coat" from 1-4 days after dosing in several animals in the high dose group (autoclaved and non-autoclaved test material). Plate counts showed that the autoclaving method killed the active bacterial ingredient.

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist RDS 007788
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C) RE

DATA EVALUATION REPORT

STUDY TYPE: Acute pulmonary toxicity/pathogenicity study in mice

MRID NO.: 413086-03 Caswell No: 66G

TEST MATERIALS: Foil® Technical Powder and Foil® Oil Flowable.

STUDY NUMBER: Study No. 1 of Project No. L08245

SPONSOR: Ecogen, Inc.

TESTING FACILITY: IIT Research Institute

TITLE OF REPORT: EPA Subdivision M Tier I Acute Pulmonary Toxicity/ Pathogenicity Testing of Foil® Oil Flowable and Technical Biopesticides.

AUTHOR: Robert L. Sherwood, Ph.D.

REPORT ISSUED: November 27, 1989

CONCLUSION: Foil OF was not lethal for, or toxic to, mice when animals were dosed intratracheally at a dose level containing 1.5×10^6 CFU/animal. Autoclaved and non-autoclaved Foil OF at 4.7×10^7 CFU/animal was lethally toxic for mice. Autoclaved Foil Technical Powder at 8.25×10^7 CFU/mouse was not toxic, but 3/18 mice died when this test material was not autoclaved. Mice that survived dosing with Foil OF or with Foil Technical powder slowly, but steadily, cleared the active bacterial ingredient from lungs and other organs.

Classification: Acceptable.

1. STUDY DESIGN : A. Test Material: Foil Technical Powder (Lot PP0009) and Foil® Oil Flowable (Lot #179-36; Batch #023A0401). B. Test animals: CD1 mouse (from Charles River Laboratories; Portage, MI).

C. Methods: Male and female mice were dosed via the intratracheal route with a single dose of either Foil Technical Powder, Autoclaved Foil Technical Powder, or Foil OF. There were 18 animals/sex/dose group. Test material was suspended in sterile saline (0.9%) and each animal received 0.05 ml. An additional untreated (naive control) group also was maintained, and a "shelf control" group (3 animals/sex) was maintained to evaluate horizontal transfer of the test bacterium. The effectiveness of autoclaving in killing the bacterium was determined. Each animal was observed daily for clinical signs of toxicity, morbidity, and mortality. Body weights were measured at the time of dosing and then weekly, or at interim sacrifice. The active bacterial ingredient was enumerated from lungs, blood, kidney, liver, caecum, and brain from 3 animals/sex/test group at 0, 3, 7, 14, and 21 days after dosing.

A preliminary study was conducted with Foil® OF, Foil Technical Powder, and killed (autoclaved) technical powder to determine a non-lethal dose for testing.

D. Results:

1. Preliminary study

In the preliminary study, five male mice each were dosed intratracheally with 0.1 ml of Foil OF containing about $1x10^9$ CFU of B.t.k. strain 2424/ml. Two of the mice died. Three of five male mice died when dosed with a reduced volume of 0.05 ml of this Foil OF test material. To determine a non-lethal dose level for Foil OF, test groups of 5 male mice were dosed with 0.05 ml of Foil OF containing either 2x106, $2x10^7$, or $2x10^8$ CFU/ml. Mice in the highest dose group exhibited rattled respiration, rough coats, and lethargy. Mid- and low-dose group animals appeared normal. Thus, a dose level of 0.05 ml of Foil OF containing $5x10^7$ CFU/ml was selected for further studies in female mice.

Five male mice were dosed intratracheally with 0.05 ml of Foil Technical Powder containing about $2x10^9$ CFU/ml. All mice died within 1 day after dosing, apparently due to test material viscosity. Therefore, for the full study, the test material for Foil Technical Powder was diluted 1:2 prior to dosing.

2. Full Study: Foil Oil Flowable

Male mice (18/group) were dosed with 0.05 ml Foil OF containing $9.45x10^8$ viable CFU/ml or with the same material autoclaved prior to dosing. This dose level represented about $4.7x10^7$ CFU/animal. Both the non-autoclaved and autoclaved test materials were lethally toxic. All animals died within one day after dosing. Signs of toxicity prior to death included rough coats, hunched posture, labored breathing, and lethargy.

Groups of 18 female mice were dosed via the intratracheal route with 0.05 ml Foil OF containing $3.05x10^7$ CFU/ml, or with an equivalent amount of autoclaved test material. This represented about $1.5x10^6$ CFU/animal. No clinical signs of toxicity were observed in any of the test animals and the test materials had no significant effect on mean body weight gains. The active bacterial ingredient in the non-autoclaved test material was steadily, but slowly, cleared from test animal lungs. Mean bacterial numbers (reported as the log geometric mean value) were 6.02 (0 days); 5.98 (3 days); 5.39 (7 days); 4.95 (14 days); 3.87 (21 days). At days 0 and 3 after dosing, from 1.31 to 2.73 (log geometric mean) were found associated with kidney, liver, spleen, brain, and caecum. At day 7 after dosing, the bacterium was cleared from organs and blood of test animals, and only 1.67 were found in the caecum. Females dosed with the killed Foil OF preparation showed no viable bacteria associated with any organ, or body fluid at any time after dosing. Of the shelf and naive control animals, only the caecal contents of one naive control animal had detectable bacteria (log geometric mean = 1.67) at 14 days after dosing. Regression analysis allowed for the prediction that B.t.k. would be completely cleared from the test animals at 50 days after dosing.

3. Full Study: Foil Technical.

Groups of 18 male mice were dosed with either 0.05 ml of viable or killed (autoclaved) Foil Technical Powder containing 1.65×10^9 viable or killed CFU/ml. This represented a dose level of about 8.25×10^7 CFU/mouse. Three of the mice treated with viable technical grade powder died at 1 to 2 days after dosing. The mean body weight of animals in this group was slightly but significantly lower than the naive control value at 3 days after dosing; but, mean weight gains were comparable to naive controls at 7, 14, and 21 days. Clinical signs of toxicity in the males dosed with viable Foil Technical Powder were observed only at 1-2 days after dosing included rough coat (1/15 animals), hunched posture (2/15 animals) and lethargy (2/15 animals). Intratracheal dosing with the autoclaved technical powder was not lethal for test animals, and did not significantly affect mean weight gains. The only sign of toxicity observed was rough coat in 8/15 animals at 1 day after dosing, and in 1/15 animals at 2 days.

No viable B. thuringiensis var. kurstaki were detected in any organs or in blood of any animals from the naive control group, shelf control group, or in any animals from the group dosed with autoclaved technical powder. Lungs from animals dosed with the viable technical powder showed the presence of the test bacterium at 7.61 (log geometric mean) at 0 days, and from 7.37-7.03 at 3, 7, 14, and 21 days after dosing. There was a trend toward slow clearance of the bacterium from lungs. The bacterium appeared in the blood at 0 days (log geometric mean= 3.48), but not at later sampling times. Mean values of bacteria in the kidneys were 2.72 at 0 days and 1.49 at 3 days and 0 at subsequent analyses. B.t.k. was detected in the spleen and liver at all sampling times, and ranged from (log geometric mean) 2.67 to 3.47. As with the lungs, there was a slow but steady clearance of bacteria over time from the liver (i.e., sequential values from 0 days to 21 days were 3.47, 3.39, 3.29, 3.13, and 2.75). The steady clearance pattern was not so obvious from the spleen. Mean values in the caecum were 2.43 at 7 days and 2.03 at 21 days. Regression analysis allowed for the prediction that B.t.k would be completely cleared from the animals at 250 days after dosing.

E. Discussion

1. Specific protocols (e.g., test materials, necessity of testing both sexes with different test materials, range-finding studies) were discussed between R. Sherwood of the contract laboratory and R. Sjoblad as the study was in progress. The Final Study Report accurately reflects the recommendations arising from the discussions.

2. The dose levels were sufficiently high for the purposes of the study, and were appropriately set from results of the range-finding study.

3. The Registrant established that the limit of detection with the method used to enumerate B.t.k. from tissues was about 30 CFU/organ. Phenylethyl alcohol-blood agar was found to be suitable for B.t.k. enumeration from caecum and stomach. Trypticase soy agar was better for sampling of other tissues and body fluids. Bacteria could be enumerated from lung and stomach samples with fairly good reproducibility.

4. To summarize the study, Foil OF (both non-autoclaved and autoclaved dosing materials) was lethally toxic to mice when administered intratracheally at a dose level that contained 4.7×10^7 CFU/animal. When Foil OF was diluted to contain 1.5×10^6 CFU/animal, all animals survived with minimal toxicity, and bacteria were steadily cleared from organs and tissues of test animals. Non-autoclaved Foil Technical Powder was lethally toxic for 3/18 mice when administered intratracheally at a dose level containing 8.25×10^7 CFU/animal. The Foil Technical Powder was not lethal for, and was only minimally toxic to, mice.

Reviewed by: Roy D. Sjoblad, Ph.D., Microbiologist
Science Analysis and Coordination, HED (H7509C)
Secondary Reviewer: Reto Engler, Ph.D., Chief, SACB (H7509C)

DATA EVALUATION REPORT

007788

STUDY TYPE: Acute pulmonary toxicity study in mice

MRID NO.: 413086-05 Caswell No: 66G

TEST MATERIALS: Foil® Oil Flowable vehicle

STUDY NUMBER: Study No. 4B of Project No. L08245

SPONSOR: Ecogen, Inc.

TESTING FACILITY: IIT Research Institute

TITLE OF REPORT: Acute Pulmonary Toxicity Limit Testing of Cutlass® Oil Flowable Vehicle.

AUTHOR: Robert L. Sherwood, Ph.D.

REPORT ISSUED: November 27, 1989

CONCLUSION: The oil flowable vehicle was lethal for mice when animals were dosed intratracheally at a dose level of 5 mg/animal. At [redacted] 5 mg/animal the test material was lethally toxic to 2/5 males and to 1/5 females. Signs of toxicity in animals dosed with 0.5 mg included rough coat, lethargy, and labored breathing which occurred from 0 to 3 days after dosing. Mice dosed with 0.05 or 0.005 mg of the test material showed no mortality or signs of toxicity.

Classification: Acceptable.

1. STUDY DESIGN : A. Test Material: Cutlass® Blank - Oil Flowable Vehicle (Lot #178-32) B. Test animals: CD1 mouse (from Charles River Laboratories; Portage, MI). Weight at dosing: 20.0-21.9 g (males); 17.6-18.4 g (females).

C. Methods: Male and female mice were dosed via the intratracheal route with a single dose (0.05 ml) of Oil Flowable Blank. There were 5 animals/sex/dose group. Dose levels administered were 0, 5, 50, 500, and 5000 ug/animal. The Oil Flowable Blank (a grayish brown liquid suspension) wasformulated in the same manner as for Foil® Oil Flowable, except that the active bacterial ingredient was replaced [redacted] Dosing suspensions of this material were prepared by dilution in 0.9% sterile saline. Animals were observed daily for clinical signs of toxicity and mortality. Individual animal body weights were determined at the time of dosing and then at 14 days post-dosing.

D. Results: All test animals dosed with the test material at 5000 ug/animal died either at dosing or within 1 hour after dosing. Two male mice and one female mouse dosed at 500 ug/animal died within 24 hours after dosing. Necropsy done on one of the males

INERT INGREDIENT INFORMATION IS NOT INCLUDED

and on the female showed uniformly dark red lungs as the only observed gross lesion. Clinical signs of toxicity observed in one to three males dosed at 500 ug included rough coat, labored breathing, lethargy, and hunched posture. No clinical signs of toxicity were observed on day 4 through day 14. Three female mice were lethargic on day 0 and 4 females were lethargic on day 1. The only other clinical sign of toxicity observed in females was labored breathing, in 1/5 females on the day of dosing. No animals died, and no clinical signs of toxicity were observed in any animal dosed with the oil flowable vehicle at 50 or 5 ug/animal. The test material did not have any significant effect on body weight gain.

D. Discussion:

1. The Oil Flowable formulation for Cutlass® OF is the same as for Foil® OF.

2. The LD_{50} value of the oil flowable vehicle was estimated at 0.74 mg for male mice and 1.07 mg for female mice. This is equivalent to approximate LD_{50} values of 37 mg/kg for male mice and 50 mg/kg for female mice.

3. Due to the toxicity of the vehicle, SACB recommends that particle masks also be worn by applicators, mixers, and loaders, unless the Registrant can provide data that is sufficient to show that applicators, mixers and loaders will not be adversely affected from pulmonary exposure under field uses and applications.