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UNDATED

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: SACB Review of Data Submitted by Kimera Oy to Support the Registration of Mycostop (Streptomyces griseoviridis) (HED No 1-0769; Caswell No 219 B; ID No 055399-NMP-R).

TO: Susan Lewis/Carl Grable (PM-21)
Fungicide-Herbicide Branch
Registration Division (H7505C)

FROM: Cindy Schaffer, Microbiologist
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler, Ph.D, Senior Science Advisor
Health Effects Division (H7509C)

DATA REVIEW RECORD

Product Name: Mycostop
Trade Name: Streptomyces griseoviridis
ID No: 055399-NMP-R
Caswell No: 219B
HED Project No: 1-0769
MRID No:

418211-01	Product Identity and Disclosure of Ingredients
418211-02	Determination of Streptomyces griseoviridis in Cucumber and Cabbage.
418211-03	Determination of Heptaenic Antibiotic Residues in Cucumber Fruit and Dill after Mycostop Treatment
418211-04	Determination of the Antifungal Antibiotic Produced by Mycostop in the Peat
418211-05	Summary Research Report
418211-06	Manufacturing Process- Mycostop
418211-07	Unintentional Ingredients Discussion Mycostop
418211-08	Mycostop (Streptomyces griseoviridis): Determination of Viability
418211-09	Certification of Limits-Mycostop
418211-10	Determination of Color, Physical State, Odor, Density and Ph of Mycostop
418211-11	Mycostop (Streptomyces griseoviridis): Determination of Storage Stability
418211-12	Acute Oral Toxicity and Infectivity/ Pathogenicity to Rats of Streptomyces

	Pathogenicity to Rats of Streptomyces griseoviridis (Mycostop)
418211-13	Acute Dermal Toxicity to Rabbits of Streptomyces griseoviridis, (Mycostop)
418211-14	Acute Pulmonary Toxicity and Infectivity /Pathogenicity to Rats of Streptomyces griseoviridis (Mycostop)
418211-15	Acute Intraperitoneal Toxicity Limit Testing of Streptomyces griseoviridis, a Microbial Pesticide
418211-16	Irritant Effects on the Rabbit Eye of Streptomyces griseoviridis (Mycostop)
418211-17	Skin Sentization Study in the Guinea Pig (Magnusson-Kligman Maximization Test)
418211-18	Hypersensitivity Incident Reporting Mycostop

ACTION REQUESTED: SACB has been asked to review product analysis and toxicology data in support of the registration of Mycostop; Streptomyces griseoviridis , in peat, to control seed and soil borne fungal pathogens that cause damping-off disease (Rhizoctonia, Pythium and Fusarium; wilt-disease, root and foot rot.

CONCLUSION: SACB is unable to support the application for registration of Mycostop due to the following major deficiencies:
1. The Product Identification package is lacking the following information:
   a) A thorough taxonomic evaluation was not provided.
   b) The characterization and isolation of each Streptomyces griseoviridis strain was not furnished by the registrant.
   c) The biochemical, nutritional, and antibiotic production/sensitivity test procedures (and results) were not supplied.
   d) The type of [redacted] agent used in the fermentation process was not identified.
   e) The following items were not addressed: an an assessment of end-use product purity; which medium was used to analyze purity; and an explanation of the methods evaluating contamination of the final product.
   f) An explanation was not provided of how the certified ingredient limits were established.
2. The Acute Oral Toxicity/Pathogenicity study did not measure the MPCA in a quantitative fashion. A validation of the methods used to assess the sensitivity of this assay was not submitted.
3. The rabbit body weight data was not submitted as part of the Acute Dermal Toxicity study.
4. The Acute Pulmonary Toxicity/Pathogenicity study was indicative of a qualitative not quantitative measure of infectivity and clearance. A validation of the organ infectivity protocol was not provided.

SUMMARY OF DATA SUBMITTED:

Product Identification (151A-10 - 151A-16):
1. The general taxonomic data (i.e. general morphology, gram stain identification, etc.) on Streptomyces griseoviridis was not included in the package for registration. This information is necessary to support the taxonomic criteria.
2. The registrant mentions several strains of S. griseoviridis acting as active ingredients in Mycostop. These strains must be identified and characterized in order to isolate these strains from others of the same species.
3. The precise test procedures (and results) used for identification and characterization (i.e. biochemical, nutritional an antibiotic production/sensitivity) must be supplied (See Subdivision M 151A-10i).
4. The description of the manufacturing process submitted by the registrant needs to be expanded to include lot sizes manufactured.
5. The registrant must state the type of [redacted] agent used during the fermentation process.
6. The registrant must submit an explanation of how the purity of the end-use product is assessed, which medium is used to analyze purity and explain methods for evaluating contamination of the final product.
7. The registrant failed to address how the certified ingredient limits are established. Generally, 5 or more representative samples are selected for the preliminary analysis of product samples (See Subdivision D, Product Chemistry 62-1).
CLASSIFICATION: UNACCEPTABLE

Acute Oral Toxicity/Pathogenicity (152A-10):
Although Streptomyces griseoviridis was detected in the feces following administration and was cleared from the intestinal tract by day 4; the procedure evaluating organ infectivity was not a quantitative measure of the MPCA infectivity and clearance. A validation of these methods must be performed to assess the sensitivity of this assay.
CLASSIFICATION: UNACCEPTABLE

Acute Dermal Toxicity (152A-11):
Streptomyces griseoviridis was not toxic to rabbits when a single 2 g/Kg dose was administered dermally. One deficiency was noted: the rabbit body weight data was not submitted.
CLASSIFICATION: UNACCEPTABLE

Acute Pulmonary Toxicity/Pathogenicity (152A-12):
Streptomyces griseoviridis was cleared from the feces by day 21. This organism was found to be somewhat toxic to the test animal at a dose of 3×10^9 CFU/gm/animal. Although the test organism was not found in any organ 24 hours post dosing, three rats died at dosing and an additional eight rats died by day 5. Clinical signs such as pilo-erection, hunched posture, abnormal gait and lethargy were noted throughout the 22 day study. The methods used to evaluate this pulmonary challenge were indicative of a qualitative, not quantitative measure of infectivity and clearance. Also, a validation of the organ infectivity protocol must be furnished to the Agency.
CLASSIFICATION: UNACCEPTABLE

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Acute Intraperitoneal Toxicity/Pathogenicity (152A-13):
The LD_{50} of Streptomyces griseoviridis was determined to be 1306 mg/kg and 870 mg/kg in male and female mice respectively. There was a high mortality rate: 100% of the mice died when dosed with either 5000 mg/kg test material or 5000 mg/kg killed test material; 40% of male and female mice died when injected with 1000 mg/kg of the test material and 40% of the females died when given 500 mg/kg test material. There were no apparent signs of infectivity as shown by a lack of clinical signs by day 4. The large size of the organism and quantity of material given to the animals was a strong influence in the toxicity of the MPCA.
CLASSIFICATION: ACCEPTABLE

Primary Eye Irritation (152A-14):
A mild conjunctival irritation was elicited due to the administration of S. griseoviridis into the rabbits' eyes. No infectivity was noted during the seven day study. SACB suggests protective eyeware be worn during application of Mycostop.
CLASSIFICATION: ACCEPTABLE--TOX CATEGORY IV

Skin Sensitization Study (152A-15):
An overall moderate skin sentization reaction was noted in the treated guinea pigs 24 and 48 hours post test challenge.
CLASSIFICATION: ACCEPTABLE

Hypersensitivity Incidents (152A-16):
One mild skin alveolar reaction was reportedly due to possible biological dust exposure. This incident could have been avoided by using the available recommended safety equipment.
CLASSIFICATION: ACCEPTABLE

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED CS

Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Product Identity and Disclosure of Ingredients
MRID NO:	418211-01
CASWELL NO:	219B
TEST MATERIAL:	Mycostop
SYNONYMS:	Streptomyces griseoviridis
PROJECT NO:	N/A
SPONSOR:	Kemira Oy, Espoo, Finland
TESTING FACILITY:	Kemira Oy, Espoo, Finland
TITLE OF REPORT:	Product Identity and Disclosure of Ingredients-Mycostop
AUTHOR(S):	Heikki Pulkkanen
STUDY COMPLETED:	December 1990
CONCLUSION:	UNACCEPTABLE: A number of deficiencies were noted including a lack of taxonomic data, test procedures (and results) used for the identification and characterization of the organism (i.e. biochemical, nutritional and antibiotic sensitivity tests) were not submitted, the description of the manufacturing process was too vague, lot size should be addressed, the type of [redacted] agent was not mentioned, a description of how the purity and/or contamination of the end-product was not addressed and finally, the registrant must submit data explaining how the certified ingredient limits were established.

PRODUCT ANALYSIS

151A-10 Product Analysis and Disclosure of Ingredients

Identity: The active ingredient of Mycostop is a bacterium identified as Streptomyces griseoviridis.

Confidential Statement of Formula has been submitted. Mycostop contains the active ingredient, Streptomyces griseoviridis (30%), [redacted]

Information on Active Ingredient:

General Taxonomy:
Taxonomy was not submitted by registrant.

History:
Streptomyces griseoviridis is a naturally occuring soil organism indigenous to Finland. It was initially isolated from Finnish Sphagnum peat.

Active ingredient characterization:

Biochemical/Nutritional Characteristics:
was not submitted by registrant.

Response of S. griseoviridis to various antibiotics:
was not submitted by registrant.

Antifungal Antibiotic Production:
Streptomyces griseoviridis produces a heptaenic compound resembling amphotericin and candicidin. A study evaluating the quantity of candicidin released from Mycostop, in steam sterilized peat, was submitted by the registrant. Candicidin reference standards (Dumex, 1366 IU/mg) were used for the calibration. This study indicated that after six weeks, 3.2 IU of candicidin was recovered from peat treated with 2 g/l Mycostop.

Inert Ingredients:

[redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Impurities:
All components used in the fermentation medium and formulation ingredients are of food grade quality; therefore, the impurities found in the raw material are considered insignificant.

Manufacturing Process(151A-11):

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Unintentional Ingredients (151A-12):

Impurities are deemed insignificant due to the food grade purity of the fermentation and formulation components.

Biological dust emitted during the grinding and packing of the dry end-product may cause hypersensitivity in certain individuals. The use of safety equipment should lower this potential risk.

The antibiotics secreted by S. griseoviridis are non-toxic topically and orally (See Acute studies 152A-10, 11); and the registrant claims the antibiotics are poorly absorbed by the gastro-intestinal tract.

Analysis of Samples (151A-13):

The concentration of S. griseoviridis is determined by standard microbiological techniques.

Product Stability/Viability:

Mycostop is considered stable for a minimum period of 6 months with a viability of between 10^8 and10^9 cfu/gm material when stored below 8 C.

Quality Control:

Each production batch is monitored for microbial purity using gram stains, microscopic analysis and culture on potato dextrose agar plates. Abnormal fermentation behavior also reveals contamination.

The viability and purity of the end-use product is determined by analyzing colony forming units/gram of Mycostop from each batch of material. Possible contaminants are indicated by the formation of colonies on the plates.

A sample of this product is registered with American Type Tissue Collection.

Certification of Limits(151A-15):

The formulated product is reported to contain between 1 x 10^8 CFU/gm and 1 x 10^9 CFU/gm.

Physical/Chemical Properties (151A-16):

	Technical Grade A.I.	End Use Product
Color	brownish-tan	brownish-tan
Physical State	finely divided powder solid at room temp.	finely divided powder solid at room temp.
Odor	fishy, rancid, sweaty	fishy, rancid, sweaty
Density	28.68 lb/ft^3	28.68 lb/ft^3
pH	1% Slurry = 5.63 10% Slurry = 5.17	1% Slurry = 5.63 10% Slurry = 5.17
Temp. Stability	below 8 C	below 8 C
Storage Stability	> 10^8 cfu/g at 6 months	> 10^8 cfu/g at 6 months
Corrosion	N/A	N/A

DISCUSSION

The product identification package was deficient on several counts as follows:

1] The general taxonomic data (i.e. general morphology, gram stain identification, etc.) on Streptomyces griseoviridis was not included in the package for registration. This information is necessary to support the taxonomic criteria.

2] The registrant mentions several strains of S. griseoviridis acting as active ingredients in Mycostop. These strains must be identified and characterized in order to isolate these strains from others of the same species.

3] The precise test procedures (and results) used for identification and characterization (i.e. biochemical, nutritional and antibiotic production/sensitivity) must be supplied (See Subdivision M 151A-10i).

4] The description of the manufacturing process submitted by the registrant needs to be expanded to include lot sizes manufactured.

5] The registrant must state the type of [redacted] agent used during the fermentation process.

6] The registrant must submit an explanation of how the purity of the end-use product is assessed, which medium is used to analyze purity and explain methods for evaluating contamination of the final product.

7] The registrant failed to address how the certified ingredient limits are established. Generally, 5 or more representative samples are selected for the preliminary analysis of product samples (See Subdivision D, Product Chemistry 62-1).

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Oral Toxicity/Pathogenicity-Rat(152A-10)
MRID NO:	418211-12
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	89953D/RKY 114/O/AC
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Huntingdon Research Centre Ltd., Cambridgeshire England
TITLE OF REPORT:	Acute Oral Toxicity/Pathogenicity to Rats of Streptomyces griseoviridis (Mycostop)
AUTHORS(S):	David J.N. Hossack, Martin N. Baker, Stuart Denton
STUDY COMPLETED:	27 June 1989
CONCLUSION:	Although Streptomyces griseoviridis was detected in the feces following administration and was cleared from the intestinal tract by day 4; the procedure evaluating organ infectivity was not a quantitative measure of the MPCA infectivity and clearance. A validation of these methods must be performed to assess the sensitivity of this assay.
CLASSIFICATION:	UNACCEPTABLE- The evaluation of organ infectivity was not a quantitative measure of microbial clearance. A validation must be performed to test the sensitivity of the method used to identify infectivity and clearance of the microbial pest control agent.

I. STUDY DESIGN

Test Material: The fungal pesticide control agent is Streptomyces griseoviridis (Mycostop). A concentration of 10% w/v of the biofungicide in sterile distilled water was prepared and administered in a volume of 20 ml/kg; equivalent to 3 x 10^9 colony forming units (CFU) per animal.

Test Animals: Twenty one male and twenty one female CD rats, approximately 4 to 6 weeks old, were obtained from Charles River, France. The male rats weighed between 99g and 122g and females weights ranged from 101g to 133g at the beginning of the study.

Methods: The animals were assigned as follows: The undosed control (UC) animal group was comprised of five male and five female rats; the autoclaved control

(AC) group contained five female and five male rats. Eleven male and eleven female rats were treated with the test substance. The rats were randomly weighed before initial dosing, 24 hours post dosing, and weekly thereafter. An electronic thermometer was used to record rat body temperature on day 1 prior to dosing, and 2, 4 and 24 hours post dosing. Treated animals were observed for signs of toxicity at frequent intervals during the first 6 hours post dosing and twice daily thereafter. The urine and feces from all rats in Group D were collected on days 2, 3, 4 and 22. All rats of each sex in the treatment groups were sacrificed by cervical dislocation. The treated animals were divided into groups based on sacrifice times as follows: Groups A, B and C, consisted of two male and two female rats each; to be sacrificed on day 2 (24 hours after dosing), day 8, and day 15 respectively. Group D is comprised of five male and five female rats which were sacrificed day on 22. Five rats per sex from the UC and AC groups were sacrificed on day 22, the last day of the study. The animals were examined by necropsy for any macroscopic abnormalities. Samples of the brain, heart, lungs, liver, kidneys, spleen, mesenteric lymph nodes as well as the contents of the stomach, small intestine (1st and 7th loop) and caecum were taken. The number of viable CFU observed in the initial dose, feces, stomach, small intestine and caecum contents was determined by diluting 1 gm of S. griseoviridis in 9 ml phosphate buffered saline (PBS) then serially diluting this mixture 1:10 in PBS. One ml aliquots of each dilution were added to molten Bacto Actinomycete Isolation Agar (BAI) and plated out in triplicate. The plates were incubated at 30.0 C for 48 hrs. The presence of the test substance in urine was determined by adding 0.1 ml urine directly to molten BAI medium, plating out in triplicate and incubating at 30.0 C for 48 hrs. To analyze the blood and organs for S. griseoviridis, 0.1 ml of blood was spread over the surface of BAI plates; and each organ was sliced in half then the freshly cut organs was smeared over the surface of BAI agar. These samples were also incubated at 30.0 C for 48 hrs. The plates were then examined for typical S. griseoviridis colonies.

II. RESULTS

A. Body Weights:

The mean body weight gain for male and female treated rats remained consistant with the AU and UC rats through the 15th day of the study. A slight

decrease in body weight gain was seen in the treated group between days 15 and 22.

B. Body Temperature:

A pyrogenic response was not indicated in male or female rats from either the treated group or the autoclaved control group.

C. Clinical Observations:

All rats receiving the test material or autoclaved test material had signs of pilo-erection on day 1. By day 3, all rats displayed normal clinical signs.

D. Necropsy observations:

No abnormalities were observed in any rat upon necropsy.

E. Microbial clearance/infectivity:

Clearance and infectivity were evaluated in the brain, heart, blood, lymph nodes, kidney, liver, spleen, lungs, feces, urine, stomach, 1st loop and 7th loop of the small intestine and caecum. The submitted data (see below) demonstrated that isolated incidences of the organism were detected in the 7th loop of the small intestine, caecum and feces 24 hrs post dosing. Clearance was demonstrated by day 3.

ORGAN	DAY	# rats*	CFU/GM OF TISSUE
7th loop (sm intestine)	2	1/4	48
caecum	2	4/4	1.45×10^4 (mean)
feces	2	10/10	5.97×10^5 (mean)
	3	4/10	3.43×10^3 (mean of 4 values)

* # of (+) treated rats/ total # of treated rats

III. SACB DISCUSSION:

When compared to controls, the treated rats exhibited a slight decrease in body weight gain from days 15 to 22. The test organism was recovered from the 7th loop of the intestine, caecum and feces in a few isolated instances. The test animal showed a distinct clearance pattern from the above organs by day 3. Although a pattern of clearance was suggested by the qualitative method used, the procedures used for evaluating organ infectivity and clearance were not adequate to fully evaluate this study. Each organ needs to be weighed and homogenized to get a quantitave analysis of the microbial load and clearance pattern.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Dermal Toxicity Test-Rabbits(152A-11)
MRID NO:	418211-13
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	89892D/RKY 114/1/AC
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Huntington Research Centre Ltd., Cambridgeshire England
TITLE OF REPORT:	Acute Dermal Toxicity Test to Rabbits of Streptomyces griseoviridis, (Mycostop)
AUTHOR(S):	Michael P. Liggett
STUDY COMPLETED:	28 June 1989
CONCLUSION:	Streptomyces griseoviridis was not toxic to rabbits when a single 2 g/kg dose was administered dermally. One deficiency was noted: the rabbit body weight data was not submitted.
CLASSIFICATION:	UNACCEPTABLE- The registrant failed to submit rabbit body weight data (See Subdivision M 152-11).

I. STUDY DESIGN

Test Material: The microbial pesticide control agent, a biofungicide, is Streptomyces griseoviridis. The absorption and stability of the test article was not determined. The potency was determined by suspending a 1 g sample of S. griseoviridis in 9 ml sterile phosphate buffered saline (PBS), making 1:10 serial dilutions and adding a 1 ml aliquot of each dilution to molten Bacto Actinomycete Isolation agar (BAI) in triplicate and incubating for 48 hours at 30 C. The final concentration was determined to be 1.46×10^9 colony forming units (CFU)/gm.

Test Animals: Five male and five female New Zealand White strain rabbits, approximately 10 to 11 weeks of age, were obtained from A. Smith, Warlingham, Surrey, England. The rabbits weighed between 2.3 and 2.5 kg at dosing.

Methods: Approximately 24 hours prior to testing, no less than 10% of the trunk fur was clipped. The biofungicide was administered over the prepared skin followed by the addition of 1.0 ml/kg bodyweight of distilled water. Elastoplast, an elastic adhesive dressing was placed over the treated site and

backed with "sleek" plaster. Approximately 24 hours following application, all dressings were removed from the animal and the skin was washed with water to remove excess test material. The rabbits were observed for clinical signs of toxicity on day 1, post dosing and twice daily during the subsequent 15 day observation period. The treated area of skin was examined 30 minutes after removal of dressing, on day 2 and daily thereafter. All animals were sacrificed on the last day of the study. A gross necropsy, including examination of abdominal and thoracic cavities was performed.

II. RESULTS

A. Body Weights:
Data on body weights was not submitted by the registrant.

B. Clinical observations:
No clinical signs of toxicity were reported.

C. Necropsy observations:
No abnormalities were observed in any rabbit upon necropsy.

III. SACB DISCUSSION:

No clinical signs of toxicity were noted. No other gross lesions were noted. One deficiency was noted: The registrant failed to submit any body weight data for review and analysis (see Subdivision M 152A-11).

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED CS
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED JTMc

STUDY TYPE:	Acute Pulmonary Toxicity/Pathogenicity-Rat (152A-12)
MRID NO:	418211-14
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	89954D/RKY 114/0/AC
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Huntington Research Centre Ltd., Cambridgeshire England
TITLE OF REPORT:	Acute Pulmonary Toxicity and Infectivity/Pathogenicity Study to Rats of Streptomyces griseoviridis (Mycostop)
AUTHORS(S):	David J.N. Hossack, Martin N.Baker, Stuart Denton
STUDY COMPLETED:	29 June 1989
CONCLUSION:	Streptomyces griseoviridis was cleared from the feces by day 21. This organism was found to be somewhat toxic to the test animal at a dose of 3 x 10^9 CFU/gm/animal. Although the test organism was not found in any organ 24 hours post dosing, three rats died at dosing and an additional eight rats died by day 5. Clinical signs such as pilo-erection, hunched posture, abnormal gait and lethargy were noted throughout the 22 day study. The methods used to evaluate this pulmonary challenge were indicative of a qualitative, not quantitative measure of infectivity and clearance. Also, a validation of the organ infectivity protocol must be furnished to the agency.
CLASSIFICATION:	UNACCEPTABLE- Although excessive toxicity was observed by the test organism, the organism was never recovered from the organs. No explanation was provided by the registrant. Also, a validation must be performed to test the sensitivity of the method used to identify infectivity and clearance of the microbial pest control agent.

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Streptomyces griseoviridis in sterile water. The potency of the dosing solution was calculated to be 3.0 x 10^9 colony forming units (CFU)/g. Each test

animal received a 1.2 ml/kg dose (approximately 1.9×10^8 cfu) intratracheally.

Test Animals: Twenty-eight male and twenty-eight female Sprague-Dawley rats, approximately 64 days old, were obtained from Harlan Sprague Dawley Inc. Frederick MD. The male rat's weight ranged from 254.1 gm to 308.3 gm and the female's weight ranged from 164.0 gm to 229.8 gm at the beginning of the study.

Methods: The animals were assigned as follows: The undosed control (UC) animal group was comprised of five male and five female rats; the autoclaved test material control (AC) group contained five female and five male rats. Thirteen male and thirteen female rats were intratracheally injected with the test substance. The rats were randomly weighed on the day of dosing, day 2 and weekly thereafter. An electronic intra-rectal thermometer was used to record rat body temperatures on day 1 prior to dosing, and 2, 4 and 24 hours post dosing. Treated animals were observed for signs of toxicity at frequent intervals during the first day post dosing and twice daily thereafter. The urine and feces from all rats in Group E (see animal assignments based on sacrifice times below) were collected on days 2 and 22. A blood sample (1ml) from each rat in Groups A through E was removed form the orbital sinus. All rats of each sex in the treatment groups were sacrificed by ether inhalation. The treated animals were divided into groups based on their sacrifice times as follows: Groups A through D consisted of 2 male and 2 female rats each sacrificed at 1 hour post dosing, 24 hours post dosing, 7 days post dosing and at day 15. Group E was comprised of 5 male and 5 female rats sacrificed on day 22. The animals were examined by necropsy for any macroscopic abnormalities. Samples of the brain, heart, liver, lungs, kidneys, spleen, mesenteric lymph nodes as well as the caecum contents were taken. The number of viable colony forming units (CFU) observed in the initial dose, feces, caecum contents and lungs was determined by diluting 1 gm of S. griseoviridis (or 1 gm feces, caecum or macerated lungs) in 9 ml phosphate buffered saline (PBS) then serially diluting this mixture 1:10 in PBS. One ml aliquots of each dilution were then added to molten Bacto Actinomycete Isolation Agar (BAI) and plated out in triplicate. The plates were incubated at 30 C for 48 hrs. The presence of the test substance in urine was determined by adding 0.1 ml urine directly to the molten BAI medium, plating out in triplicate and incubating at 30 C for 48 hrs. To analyze the blood and organs for the presence of S. griseoviridis, 0.1 ml blood was spread over the

surface of BAI plates; and each organ was sliced in half, then the freshly cut organ was smeared over the surface of BAI agar. These samples were also incubated at 30 C for 48 hrs. The plates were then examined for typical S. griseoviridis colonies.

II. RESULTS

A. Body Weights:

One female rat from Group E demonstrated a loss in weight from day 15 to day 22. No other body weight changes were noted.

B. Body Temperature:

A pyrogenic response was not indicated in male or female rats from either the treated groups or the controls.

C. Clinical Signs:

All rats in Groups A through F showed signs of pilo-erection, hunched posture, and an abnormal gait. Lethargy was exhibited in all rats of Groups B through F except all males from Group E. A decreased respiratory rate was noted in three female rats, one from Group B, one from Group D and one in Group E. One male also showed a decreased respiratory rate in Group E. The following animals had a pallor of the extremities: one Group B female, two males and one female from Group D and one Group E female.

D. Mortality:

Three rats died at dosing, which were subsequently replaced with an additional three rats. One Group C female died 1/2 hour post dosing. Two males and one female from Group D and one Group E female was found dead day 2. One Group C female died on day 3; and by day 5 another Group E female died.

E. Microbial clearance/infectivity:

Clearance and infectivity were evaluated in the brain, blood, lymph nodes, kidney, liver, spleen, lungs, caecum, feces and urine. The average CFU/gm feces (in six rats) at 24 hrs post dosing was 1.57×10^4. At 21 days the CFU/gm was less than 1000. S. griseoviridis was also present in the caecum contents at approximately 1.2×10^3 CFU/gm in two rats 24 hrs after dosing. At 1 hour post dosing, 1.50×10^6 CFU was detected in the lungs of one animal. Less than 1000 CFU test material per pair of lungs was recovered from any other rat at 1 hour post dosing. After 24 hours, less than 100 CFU test material per pair of lungs were detected in any Group B rat. The organism was not recovered in any other organ.

F. Necropsy observations:

A slight lung congestion was discovered in one

Group D male. One Group C female had a brown fluid and food in the thoracic cavity and yellow fluid in the small intestines. No other abnormalities were observed upon necropsy.

III. SACB DISCUSSION:

One rat exhibited a weight loss on day 22 of the study. All rats from Group A through F displayed signs of pilo-erection, hunched posture, and an abnormal gait. All rats from Groups B through F experienced lethargy except the males in Group B. A decreased respiratory rate and pallor of the extremities were noted in three females (one each) from Groups B, D, and E. One male from Group E also had a lowered respiratory rate. Pallor of the extremities was also noted in two male rats from Group D. Eleven rats died during the study. Three died immediately post dosing, one died 1/2 hour post dosing, four on day 2, one day 3 and one day 5. The necropsy of these animals revealed one male with congested lungs and one female had brown fluid and food in the thoracic cavity along with yellow fluid in the small intestine. The average CFU/gm in the feces (in six rats) at 24 hrs post dosing was 1.57×10^4. The test material was not detected from the feces by day 21 of the study. Two rats presented 1.2×10^3 CFU/gm from the caecum contents at 24 hrs post dosing. In one animal, a detection level of 1.5×10^6 CFU/gm was found in the lungs at 1 hour post dosing. The other animals revealed less than 1000 CFU in the lungs. The organism was not detected in any of the organs. The following deficiencies were noted:

1. The clinical toxicity was excessive considering the MPCA was not recovered from any organ. The registrant should provide an explanation for this toxicity and for their absence in the organs examined.
2. A validation of the organ infectivity protocol must be furnished. The methods used were indicative of a qualitative, not quantitative, measure of infectivity and clearance.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute Intraperitoneal Toxicity/Pathogenicity-Mouse (152A-13)
MRID NO:	418211-15
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	L08279
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	IIT Research Institute, Chicago IL
TITLE OF REPORT:	Acute Intraperitoneal Toxicity Limit Testing of Streptomyces griseoviridis, a Microbial Pesticide
AUTHORS(S):	Robert L. Sherwood, Ph.D.,
STUDY COMPLETED:	30 May 1990
CONCLUSION:	The LD_{50} of Streptomyces griseoviridis was determined to be 1306 mg/kg and 870 mg/kg in male and female mice respectively. There was a high mortality rate: 100% of the mice died when dosed with either 5000mg/kg test material or 5000mg/kg killed test material; 40% of male and female mice died when injected with 1000mg/kg of the test material and 40% of the females died when given 500mg/kg test material. There were no apparent signs of infectivity as shown by a lack of clinical signs by day 4. The large size of the organism and quantity of material given to the animals was a strong influence in the toxicity of the MPCA.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Streptomyces griseoviridis in 0.9% sterile saline. Each test animal received a dose of 0.02 ml per gram of body weight.

Test Animals: Thirty male and thirty female CD1 mice, approximately 5 weeks old, were obtained from Charles River Laboratories, Portage MI. The male mice weight ranged from 22.3 gm to 23.1 gm and the females weight range from 18.8 gm to 19.8 gm at the beginning of the study.

Methods: Six groups containing five male and five female mice were catagorized based upon the dose (mg/kg body weight) of test material administered. The groups consisted of the following: Group 1

received a dose of 5000 mg/kg (3.06×10^7 - 3.86×10^7 CFU), Group 2 received 1000 mg/kg (5.47×10^6 - 7.41×10^6 CFU), group 3 was given 500 mg/kg (2.9×10^6 - 3.86×10^6 CFU), group 4 was administered 100 mg/kg (5.8×10^5 - 7.73×10^5 CFU), the Killed Test Article control group (KTA) a dose of 5000 mg/kg autoclaved test material and an undosed control group (NC). The mice were randomly weighed before initial dosing and weekly thereafter. Treated animals were observed for signs of toxicity on a daily basis. A limited necropsy was performed on all mice which showed clinical signs or died during the study. The MPCA enumeration was carried out by plating serial dilutions of dose 1 on potato dextrose agar (PDA) in duplicate. Plates were incubated for at least 4 days at room temperature.

II. RESULTS

A. Body Weights:

The mean body weights of all rats remained consistant throughout the study.

B. Clinical observations:

The following clinical signs were observed:

Observation	Sex	Group #	Day	# mice*
Rough Hair Coat	M & F	NC	0	10/10
	"	1	"	9/10
	"	2	"	10/10
	"	3	"	10/10
	"	4	"	10/10
	"	KTA	"	10/10
	F	NC	1	5/5
	M & F	2	"	10/10
	"	3	"	10/10
	M	4	"	5/5
	"	KTA	"	4/5
	F	KTA	"	5/5
	M	2	2	3/4
	F	2	"	3/3
	M	4	"	1/4
	M & F	4	3	6/6
	F	2	4	3/3
Animals Found Dead	F	1	0	1/5
(or Near Death)	M	NC	1	1/5
	M & F	1		9/9
	M	2		1/5
	F	2		2/5
	M	KTA		2/5
	F	KTA		3/5
	M	2	2	1/4
	F	3		2/5
	M & F	KTA		6/6
Lethargic	M	1	0	5/5
	F	1		4/5
	M	KTA	2	1/4

(Cont.)	Sex	Group #	Day	# mice*
Labored Respiration	F	2	1	1/5
	F	2	2	1/3
Ocular Discharge	F	2	1	1/5
	F	2	2	1/3
Closed Eyes	M	KTA	1	2/5
	F	KTA	1	2/5
	M	KTA	2	1/4
Distended Abdomen	F	3	7-14	1/3
Hunched Posture	M	KTA	2	1/4
Loose Stool	M	KTA	2	1/4

*number of mice with clinical signs/number of mice in the test group.

D. Necropsy Evaluations:

The following is a summary of the post mortem examination findings:

NC mice: Day 1: one mouse was cannibalized.

Dose 1 mice: Day 1: ^All mice except 1 showed signs of autolysis.
^The test material appeared in the abdominal cavity of one mouse.
^One mouse had a mottled black liver.
^Two dark red lines appeared on the surface of the lung lobes of one mouse.
^One mouse had mottled red lungs with red fluid in the pleural cavity.
^A (1mm) white lesion on the liver was noted in one mouse.
^Mottled red lungs and a lesion at the dosing site was found in one mouse.

Dose 2 mice: Day 14: One mouse had an enlarged spleen and masses in the abdominal cavity, and a subcutaneous lesion on the abdomen.

Dose 3 mice: Day 2: Mottled red lungs appeared in one mouse while another showed signs of autolysis
Day 14: One mouse had an enlarged spleen and masses in the abdominal cavity.

KTA mice: Day 0-2: All mice showed signs of the test substance in the abdominal cavity.

III. RESULTS:

The body weight gain of all mice in this study remained consistant throughout the study. Clinical observations revealed a rough hair coat in the majority of mice on the first 2 days of the study. Lethargy was noted in all but one mouse from group 1 mice on day 1. One mouse showed signs of a distended abdomen from days 7 through 14. Other clinical signs such as hunched posture, closed eyes, labored respiration, ocular discharge, subcutaneous lesion were noted in isolated instances. 100% of the males and females in test group 1 and the KTA group spontaneously died while 40% were found dead in group 2. The mortality of the group 3 female mice was also

40%. During necropsy, autolysis was revealed in all animals except one from the dose 1 group. Other observations from the dose 1 group were as follows: one mouse had a black mottled liver, one had dark red lines across the surface of the lung lobes, two had mottled red lungs, one with a red fluid in the pleural cavity and the other had a small lesion at the dosing site, and another mouse had a white (1mm) lesion on its liver. The MPCA was found in the abdominal cavity of all KTA mice. The day 2 necropsy revealed one dose 3 animal with mottled red lungs and the other (dose 3) was too autolysed to note observations. One dose 2 female and one dose 3 female were examined for necropsy on day 14. Both had enlarged spleens and abdominal masses. The dose 2 female also had a lesion on the abdomen. The spleens and masses were evaluated for the test substance by plating on PDA. The growth of the test article was not evident.

IV. SACB DISCUSSION:

The LD^{50} of S. griseoviridis by intraperitoneal administration was determined to be 870 mg/kg and 1306mg/kg in female and male mice respectively. Since all clinical signs were resolved by day 3, this organism was not considered infective. The toxicity was possibly due to the excessive quantity and large size of S. griseoviridis.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Primary Eye Irritation Study-Rabbits (152A-14)
MRID NO:	418211-16
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	891076D/RKY 114/4/SE
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Huntington Research Centre Ltd., Cambridgeshire England
TITLE OF REPORT:	Irritant Effects on The Rabbit Eye of Streptomyces griseoviridis (Mycostop)
AUTHOR(S):	Michael P. Liggett, David J. Hossak, Martin Baker
STUDY COMPLETED:	13 June 1989
CONCLUSION:	A mild conjunctival irritation was elicited due to the administration of S. griseoviridis in the rabbits' eyes. No infectivity was noted during the seven day study. SACB suggests protective eyeware be worn during application of Mycostop.
CLASSIFICATION:	ACCEPTABLE--TOX CATAGORY IV

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Streptomyces griseoviridis. The absorption and stability of the test article was not determined. The potency was determined by suspending a 1 g sample of S. griseoviridis in 9 ml sterile phosphate buffered saline (PBS), making 1:10 serial dilutions and adding a 1 ml aliquot of each dilution to molten Bacto Actinomycete Isolation agar (BAI) in triplicate and incubating for 48 hours at 30 C. The final concentration was determined to be 1.46 x 10^9 colony forming units (CFU)/gm.

Test Animals: Six male New Zealand White strain rabbits, approximately 13 to 14 weeks of age, were obtained from A. Smith, Warlingham, Surrey England. The rabbits weighed between 2.9 and 3.3 kg at dosing.

Methods: Approximately 100 mg (1.46 x 10^8 CFU) of Streptomyces griseoviridis was administered into the conjunctival sac of one eye in each rabbit. The eyelids were then held together for one second. The other eye served as a control and remained untreated. The eyes were examined at 1 hour post

dosing, and at 1, 2, 3, and 7 days after inoculation. A "handheld torch" was used to aid in the observation of the eyes. All animals were observed for ill health or toxicity on a daily basis. To comply with U.K. animal welfare guidelines, one animal was treated in advance of the others. The ocular lesions were graded and scored according to the Draize method as described in Subdivison M (152 A-14). The Rabbit's eyes and eyelids were examined for the presence of bacterial spores by swabbing conjunctival fluid from the lower lid, canthus, and outer lids with sterile saline moistened cotton wool swabs. Swabs were taken before the initial dose and at 1, 2, 3, 4 and 7 days post dosing. BAI plates were directly inoculated with the swabs and bacterial colonies were counted after incubation.

II. RESULTS

A. Conjuctivae:

All 6 rabbits showed slight signs of redness, swelling and discharge at 1 hour post dosing. The first day after instillation, slight redness and discharge were noted in 5/6 rabbits. The conjunctivae appeared normal by day 2.

B. Cornea:

The cornea of all rabbits appeared normal throughout the course of the study.

C. Iris:

No signs of inflammation were noted.

D. CFU from conjunctival swabs of treated eyes:

hours post dosing	site	#(+)rabbits/6	Ave # CFU
24	lids	3	6.7
	fluid	4	20.0
48	lids	2	3.0
		1*	19.0
	fluid	5	22.8
72	lids	3	1.0
	fluid	1	7.0

* One rabbit showed a positive result in a control lid.

III. SACB DISCUSSION:

A mild transient conjunctival irritation was elicited by a single installation of S. griseoviridis into the rabbits' eyes. The test substance was not detected in any animal at 96 hours post administration.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Skin Sensitization Study-Guinea Pig (152A-15)
MRID NO:	418211-17
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	5383-520/18R
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Hazleton UK, North Yorkshire England
TITLE OF REPORT:	Skin Sensitization Study in the Guinea Pig (Magnusson-Kligman Maximization Test)
AUTHOR(S):	F. Lackenby, B.SC.
STUDY COMPLETED:	February 1991
CONCLUSION:	An overall moderate skin sentization reaction was noted in the treated guinea pigs 24 and 48 hours post test challenge.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The microbial pesticide control agent is Streptomyces griseoviridis. The absorption, stability and potency of the test article was not determined. A preliminary study was performed to determine the highest non-irritant concentration and threshold irritation concentration to be used for the topical induction and challenge applications. Concentrations of 100%, 50%, 25% and 10% w/v test material was applied to exposed test sites. Based on the results of this study, a concentration of 50% w/v in distilled water was used for both the topical induction and challenge applications.

Test Animals: Forty female albino Dunkin-Hartley guinea pigs were obtained from SF Animal Supply Ltd., Henfield, England. The guinea pigs weighed between 445 and 613 grams at dosing.

Methods: The guinea pig maximization test consists of two stages. The induction phase, which consists of a series of intradermal injections potentiated by a simultaneous injection of Complete Adjuvant (50% v/v in distilled water), followed 7 days later by a topical application of the test material. Three sets of intradermal injections were made on either side of a 4cm x 5cm clipped area on the guinea pigs shoulder as follows:

1. 0.1 ml 5.0% w/v Mycostop in distilled water
2. 0.1 ml Complete Adjuvant (50% v/v in water)

3. 0.1 ml 5.0% w/v Mycostop in distilled water plus 0.1 ml Complete adjuvant (50% v/v in water)

Twenty guinea pigs in the control group received a similar set of injections at either side of the midline as follows:

1. 0.1 ml Distilled water
2. 0.1 ml Complete Adjuvant (50% v/v in water)
3. 0.1 ml Complete Adjuvant (50% v/v in water).

The same shoulder area was clipped six days following the intradermal induction. A 2 cm x 4 cm patch of Whatman No. 3 filter paper saturated with 50% w/v test material (0.5 ml) was applied to the clipped area and fastened by an overlapping occlusive tape. The control animals received a similar treatment using "vehicle" only. The test and control guinea pigs flanks were shaven on day thirteen after the induction phase. The next day (day 14), these animals were challenged with an application of 50% w/v test material (0.5 ml) applied to the left flank of each guinea pig on a "Hill Top Chamber". Distilled water was applied to the other flank in the same fashion. After a contact period of twenty four hours, the occlusive dressings were removed. The skin reactions at the challenge site were assessed twenty four and forty eight hours after the dressings were removed and graded according to the following scale:

REACTION	VALUE
No reaction	0
Slight patchy redness	0.5
Slight, but confluent or moderate patchy redness	1
Moderate redness	2
Severe redness and swelling	3

NOTE: Slight patchy redness is considered within background levels and therefore not considered a response.

The degree of sensitization potential was determined according to the percentage of animals showing a positive response as follows:

% Animals responding	# Animals in test group	Classification
0	0	non-sensitizer
5 - 10	1 - 2	weak sensitizer
15 - 35	3 - 7	mild sensitizer
40 - 60	8 - 12	moderate sensitizer
65 - 80	13 - 16	strong sensitizer
80 -100	17 - 20	severe sensitizer

II. RESULTS

A. Skin Reactions in Test Animals after challenge application:
At 24 hrs:

Four treated and two control guinea pigs experienced slight patchy redness after challenge with the test material. Two control guinea pigs also showed signs of slight patchy

redness when challenged with water alone. Three treated animals showed signs of slight but confluent or moderately patchy redness after a challenge with the test material. The test material challenge also proved positive for two other guinea pigs, one experiencing moderate redness while the other had severe redness and swelling.

B. Skin Reactions in Test Animals after challenge application at 48 hours:
Two control guinea pigs showed signs of slight patch redness; one when challenged with the test material while the other was challenged with water. Four treated animals had slight, but confluent or moderately patchy redness and seven treated guinea pigs experienced moderate redness after the test material challenge.

II. SACB DISCUSSION:

At 24 hours post test material challenge, 25% (5/20) of the animals showed a mild sensitization reaction; while at 48 hours, a moderate skin sensitization reaction was noted in 55% (11/20) of the guinea pigs. No specific reactions were noted in the control animals. An overall moderate skin sensitization reaction was noted in the treated guinea pigs 24 and 48 hours post test challenge.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: J.Thomas McClintock, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Hypersensitivity Incident Report (152A-16)
MRID NO:	418211-18
CASWELL NO:	219B
TEST MATERIAL:	Streptomyces griseoviridis
SYNONYMS:	Mycostop
PROJECT NO:	N/A
SPONSOR:	Kemira Oy, Espoo Finland
TESTING FACILITY:	Kemira Oy, Espoo Research Centre, Espoo Finland
TITLE OF REPORT:	Hypersensitivity Incident Reporting Mycostop
AUTHOR(S):	Heikka Pulkkanen
STUDY COMPLETED:	August 1990
CONCLUSION:	One mild skin alveolar reaction was reportedly due to possible biological dust exposure. This incident could have been avoided by using the available recommended safety equipment.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Hypersensitivity Incidents:
A mild alveolar reaction was reported in one part-time summer trainee in 1986. An examination at the Institute of Occupational Health revealed the reaction was possibly due to biological dust exposure. The employee recovered completely. This incident could have been avoided by using the available recommended safety equipment. All further incidents of hypersensitivity incidents must be reported to the Agency.