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

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SEP 13 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Review of Toxicology and Product Analysis Data in Support of an Experimental User Permit (EUP) Application of Mycoleptodiscus terrestris, Submitted by Ecoscience Laboratories, Inc. for Control of Eurasian Watermilfoil

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

From: Rita Briggs, Ph.D., Chemist RB.
Science Analysis and Coordination Branch (SACB)
Health Effects Division (H7509C)

Through: Reto Engler, Ph.D., Chief
SACB/HED

DATA REVIEW RECORD

Product Name: Mycoleptodiscus terrestris, mycelia
ID No: 064296-EUP-R
Synonym: S395004
Caswell No: 584H
HED Project: 1-1156
MRID No: 418335-01 Temperature Growth Data; Sample Analysis; Physical/Chemical Properties.
418436-02 Abbreviated Acute Oral (Conidia); 152A-10
418335-02 Acute Oral (TGAI); 152A-10
418335-03 Acute Pulmonary; 152A-12
418335-04 Acute Intraperitoneal; 152A-13
418335-05 Survey of Hypersensitivity Incidents; 152A-15

ACTION REQUESTED:

To review toxicology and product analysis data in support of an EUP application for use of Mycoleptodiscus terrestris mycelia to control Eurasian Watermilfoil. Field studies will be conducted on two sites (one acre/site) in each of eight states. A total of 19.2 lbs. of active ingredient per site will be used during the year 1 April 1992 to 1 April 1993. The objective is to determine application rates of the active ingredient and timing under various geographic conditions.

BACKGROUND:

Representatives of the Agency (RD, HED, EED) and EcoScience Laboratories, Inc. have previously discussed data requirements in support of an EUP and registration of Mycoleptodiscus terrestris (see correspondence: 2/9/90 memo from Sjoblad (SACB) to Lewis/Grable (RD); 2/6/90 letter E.R. Butts International Inc. to Grable; 11/1/90 letter from Green (EcoScience) to Lewis/Hutton (RD); 6/23/89 letter from E.R. Butts to Lewis; 8/16/89 memo from Hazel (HED) to Lewis). Pre-EUP meetings (11/18/90, 12/14/89, 6/8/89) also were held to discuss data requirements, with particular reference to waivers of certain toxicological studies. Based on these discussions and correspondence, the following changes in data requirements were made:

(1) 151A-12: Intraperitoneal studies on batch samples to detect unintentional ingredients. The registrant requested a waiver based on the rationale that the purity of the manufactured product will be monitored and certified, that Mycoleptodiscus terrestris does not produce toxins and growth does not occur at mammalian temperatures. Data on the effects of temperature on the growth of Mycoleptodiscus terrestris hyphae are included with the present EUP application and indicate that optimal growth occurs at 25°C, some growth at 15°C, and no discernible growth at 5, 35 or 45°C. Based on a personal communication with EPA (not identified) on November 8 (see appendix 1, Vol. 1B, Proposed Protocol....), the Agency agreed that a study to determine the maximum dose at which no intraperitoneal toxicity occurs could be conducted, and the determined NOEL be used for routine checking of production batches of M.t. for toxicity. EcoScience Laboratories, Inc. have since submitted a protocol for such a study to be reviewed by SACB. SACB's comments on the protocol are on p.23 of this report.

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(2) 151A-15: Antibiotic Resistance Studies. A waiver was initially requested for these studies based on two facts: the organism is indigenous and no antibiotic resistance gene has been introduced into its genetic material, and it is non-pathogenic. EcoScience Laboratories Inc. now proposes to conduct a study that would determine the susceptibility of M.t. to five antifungal agents. SACB's comments on EcoScience's protocol for this study are attached (p.23).

(3) According to 2/9/90 memorandum from Sjoblad (HED) to Lewis/Grable (PM-21, RD), SACB supports the following adjustments in data requirements:

152A-10: Acute Oral toxicity/pathogenicity study using the end-use product. SACB agreed to the requested waiver for this portion. An acute oral study using the a.i., however, was required and has been submitted. In addition, because of the potential of human oral exposure to Mycoleptodiscus terrestris conidia (spores are produced 4 days following application into water), an abbreviated oral study to test this form of the organism was agreed upon at a pre-EUP meeting (12/14/89) and submitted with the present application. Studies are reviewed on p. 9-13.

152A-11: Acute dermal tox/path.; this study was waived.

152A-12: Acute Pulmonary tox/path. studies testing the active ingredient and end-use product. Because of the large particulate size of the test material, SACB recommended a substitute study using a homogenized preparation of the fungal mycelium. Study was submitted and reviewed (p.14).

152A-13: Acute Intravenous toxicity study. It was agreed that an acute intraperitoneal study with the a.i. be substituted for the acute i.v. study. Study was submitted and reviewed (p.19).

152-14: Primary Eye Irritation was waived.

Note that, in general, SACB's concurrence with the above changes were made on the basis of expected routes of human exposure to Mycoleptodiscus terrestris, in accordance with the use patterns proposed in the present EUP.

In addition to the modifications mentioned above, the registrant has requested in the present submission (see Section VI, p.8 of this report) that data on specific gravity, pH, and stability to sunlight, water, and metal ions of the test material be waived. SACB's response to these requests are listed below under Conclusions and in Section VI (p.8).

CONCLUSIONS:

1. SACB can support the application for the present EUP.
2. SACB agrees with the rationale presented by the Registrant in requesting waivers for submitting data on specific gravity and stability to sunlight, water, and metal ions (see section VI, p. 7) of the test material and does not require these data to be submitted for registration. However, although the pH of the end-use product will not be required, SACB recommends that information on the pH of the fermentation material be provided.
3. The data submitted on product identity and manufacturing process are sufficient to support the request for an EUP. For registration, additional data on the analysis of samples, storage stability, and pH are required.
4. The toxicocology data indicate that Mycoleptodiscus terrestris, as tested under the conditions of the studies submitted, is non-toxic, non-pathogenic, and non-infective. SACB does not require additional data to be submitted for registration and believes that human exposure to the test material will be minimal. However, it is recommended that protective clothing, such as eye goggles, be worn during the processing and application of the test agent.
5. SACB believes that the protocols for two of the proposed three additional studies (i.e., sensitivity of M.t. to 5 antifungal agents, and storage stability at room temperature) are adequate. With respect to the proposed study to determine the limit for non-specific toxicity testing in batch samples, SACB thinks testing at the 125 mg dose level is redundant since the results from the Acute Intraperitoneal Toxicity/Pathogenicity study included in the present submission indicates that this dose level caused some mortality. It is SACB's opinion that testing at two dose levels (62.5 mg and 31.2 mg/mouse/0.5 ml) should be sufficient. Reviews of the protocols are on p.23.
6. Since the test material (Mycelium terrestris conidia) used in the Acute Oral Toxicity Study (p. 9) was reported to contain a contaminant (Bacillus), SACB questions the adequacy of the quality control procedures now in place.

SUMMARY OF REVIEWS

I. PRODUCT ANALYSIS (151A-10)

Identity: The organism is identified as Mycoleptodiscus terrestris mycelia. It has no assigned ATCC number although one has been applied for. Also, it has no assigned product or trade name. The company references are: ECO 891, MT1 and BCW 100

Confidential Statement of Formula has been submitted. The dry active ingredient contains 100% fungal mycelia, the formulated material contains 20% wet weight/weight of fungal mycelia.

Information on Ingredients:

Taxonomy: The active ingredient is identified as Mycoleptodiscus terrestris, (Gerd.) Ostazeski according to morphological features of the fungus described by B.C. Sutton (Trans. Br. Mycol. Soc. 60 (3), 525-536 (1973). A copy of the article is included in the application package.

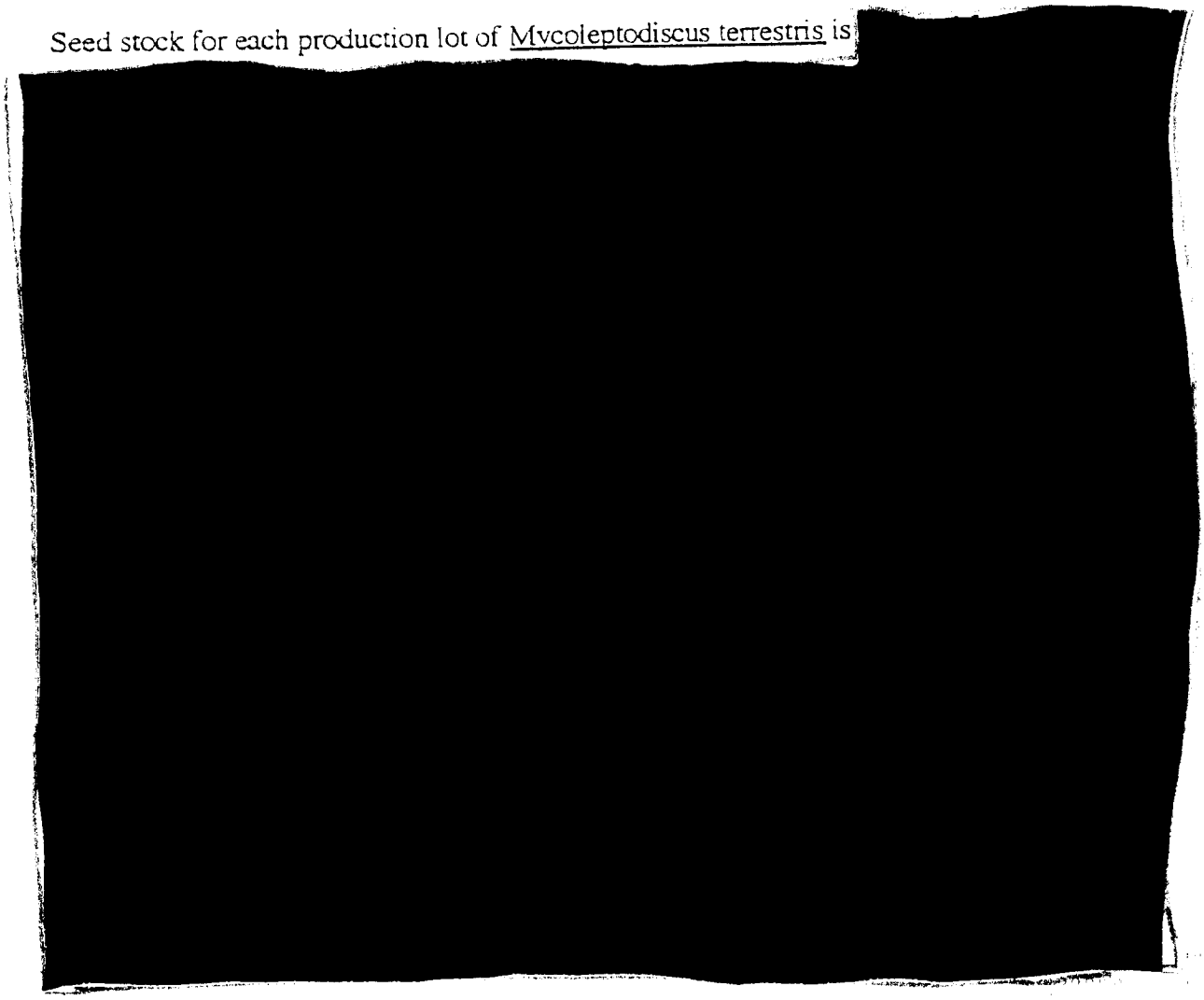
History: Mycoleptodiscus terrestris is described as a naturally occurring organism found in Massachusetts, Illinois and the Southeastern U.S. The orginal organism was isolated from Eurasian Watermilfoil (Myriophyllum spicatum) in Quabbin Reservoir, Massachusetts. Other samples have been isolated from Eurasian Watermilfoil in Florida and Michigan. There are no reports in the literature to indicate that Mycoleptodiscus terrestris is toxic or pathogenic to mammals or other animals.

Biological Properties: (a) The active ingredient is, reportedly, plant specific. The fungal mycelia supposedly attach themselves to the host plant and produce enzymes that enable it to invade and decompose plant cells. (b) There is no published literature on the life cycle of Mycoleptodiscus terrestris in aquatic systems. Experiments conducted at Ecoscience Laboratories have shown that M.t. does not grow at mammalian temperatures; optimal growth occurred at 25° C, some growth was seen at 15°C, and no discernible growth at 5, 35, and 45°C. (Vol. 2A). Also, it is reported that results from several field tests show that fragmented mycelia and formulated granule of M.t. are not detectable in water at 4-5 weeks after application. (c) M.t. strain is genetically unaltered.

MANUFACTURING PROCESS INFORMATION NOT INCLUDED

II. MANUFACTURING PROCESS ** CBI** (151A-11)

Seed stock for each production lot of Mycoleptodiscus terrestris is



III. DISCUSSION OF FORMATION OF UNINTENTIONAL INGREDIENTS : (151A-12)

It is reported that no toxins or sensitising substances are known to be produced by the active ingredient, M.t., which is an indigenous organism unaltered by genetic engineering. However, SACB raises the question of whether quality control methods are sufficient to ensure that media, production batches, and end-use product are free of contaminants. The material used in the Acute Oral Toxicity Study (p.9) was reported to contain a species of

Bacillus suggesting that a more thorough monitoring of each production batch may be needed.

IV. ANALYSIS OF SAMPLES (151A-13)

Fungal cultures which are microscopically identified as Mycoleptodiscus terrestris are grown on Rabbit Food Agar under a 14-hour photoperiod at 20-25°C until conidia are produced. Taxonomic characteristics, as described by Sulton and Alcorn (1990) in Mycological Research 94(4):546-566 (article submitted), are used to verify preliminary identification. However, it should be noted that data on analysis of samples from five batches have not been completed. These should be forwarded to the Agency for registration.

V. CERTIFICATION OF LIMITS (151A-15)

The formulated product is reported to contain $\geq 10^4$ CFU/gm of formulation and 20% wet wt/wt of fungal mycelia. Note that it is reported in this section that a method to determine the percent of dry weight has not yet been developed. However, the dry weight is reported to represent 18.3 % (Acute Oral Study), 19.2% (Acute Pulmonary Study) and between 22.3-22.6% (Acute Intraperitoneal Study) of the wet weight.

VI. PHYSICAL/CHEMICAL PROPERTIES (151A-16)

	Technical Grade a.i.	End Use Product
Color	Brown	Not available
Physical State	Solid	Solid
Odor	None	None
Storage Stability	Not available	Not available
Viscosity	----------	Solid
Miscibility	----------	Solid

Corrosion data are reported by the registrant to be non-applicable since the container will be plastic and the end-use product is dry. SACB is not requesting that corrosion data be generated. However, SACB believes that the pH of the test material ingredients (fermented material) should be determined and submitted to the Agency.

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NOTE:
A waiver has been requested for data on specific gravity on the basis that it could be variable because the fungal mycelium cake is a living organism. SACB agrees with the justification for a waiver.

A waiver has been requested for data on pH because the technical grade a.i. is a living organism and the final formulation is a solid. SACB agrees that testing pH of the final formulation is not feasible. However, SACB thinks that the pH of the fermented material could be easily determined, and this information should be sent to the Agency.

A waiver has been requested for generating data on stability to metal ions, sunlight and water since the test materials will be stored in opaque plastic containers, and the final formulation will be dry. SACB supports the request for this waiver.

Storage stability studies have just begun. Since storage data are required for registration, data should be sent to the Agency when available.

DISCUSSION
The submitted data on product identity and manufacturing process are adequate to support the proposed EUP. For registration, supplemental information on analysis of samples and storage stability need to be forwarded to the Agency when they become available. Additionally, it is recommended that data on the pH of fermented material be provided.

DATA EVALUATION REPORT (152A-10)

Reviewed by:	Cindy Schaffer, Microbiologist, SACB/HED CS
Secondary Reviewer:	Rita Briggs, Ph.D., Chemist, SACB/HED RB

Study Type:	Acute Oral Toxicity /Pathogenicity - mice
MRID No:	418436-02
Caswell No:	584H
Test Material :	Mycoleptodiscus terrestris conidia
Project No:	LO8247
Sponsor:	EcoScience Laboratories, Inc., Amherst, MA.
Testing Facility:	IIT Research Institute, Chicago, Ill.
Title of Report:	Acute Oral Toxicity Limit Testing of Mycoleptodiscus terrestris, a Fungal Herbicide.
Authors:	Robert L. Sherwood, Ph.D.
Study Completed:	12 September, 1990
Conclusion:	This is an abbreviated oral toxicity study designed to evaluate exposure to Mycoleptodiscus terrestris conidia (study recommended in pre-EUP discussions; see Background Section). Results from the present study demonstrate that during a 7-day observation period no mortality, no clinical signs of toxicity and no gross abnormalities occur when a single oral dose of 2.7×10^7 CFU of conidia is administered to mice orally .
Classification:	Acceptable.

I. STUDY DESIGN

Test Material:	Conidia of Mycoleptodiscus terrestris suspended in sterile saline at a concentration of 1.54×10^8 CFU/ml.
Test Animals:	Ten female CD1 virus antibody-free mice were obtained from Charles River Breeding Laboratories, Portage, MI. Five mice were assigned as test animals and five as naive controls. The mice were 7 weeks of age and weighed between 20-25 grams at the beginning of the study.
Methods:	Following a 24-hour fast, the animals were given, by gavage, 0.47 ml of the suspended test substance. The actual

concentration of the test substance was determined immediately following dosing by plating a sample of the dosing suspension. This test also detected the presence of any contaminants. Fifteen minutes post-dosing and on each of the succeeding days, except for day 6, the animals were examined for clinical signs of toxicity. The animals were weighed just prior to dosing and sacrifice. A gross pathological examination was conducted at the time of death.

II. RESULTS

The plating procedure indicated that the dosing suspension contained a species of Bacillus which was not fully identified. No adverse health effects resulted from oral exposure to this contaminant. The results from the actual study showed that, when a single dose (determined to be 2.7×10^7 CFU) of the test material Mycoleptodiscus terrestris is given orally, there are no clinical signs of toxicity, no mortality and no abnormalities at the time of necropsy.

III. SACB DISCUSSION

Results from the present study suggest that exposure to approximately 2.7×10^7 CFU of fungal conidia , via the oral route, does not pose any adverse health effects in mice.

DATA EVALUATION REPORT (152A-10)

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer: Rita Briggs, Ph.D., Chemist, SACB/HED

Study Type: Acute Oral Toxicity Pathogenicity - mice
MRID No: 418335-02
Caswell No: 584H
Test Material : Mycoleptodiscus terrestris , mycelia (TGAI)- SN3
Project No: LO8247 - SN3
Sponsor: EcoScience Laboratories, Inc., Amherst, MA.
Testing Facility: IIT Research Institute, Chicago, Ill.
Title of Report: Acute Oral Toxicity Limit Testing of Mycoleptodiscus terrestris, a Fungal Herbicide.
Authors: Robert L. Sherwood, Ph.D.
Study Completed: October, 1990
Conclusion: A test dose of Mycoleptodiscus terrestris mycelia (TG) at a concentration of 3×10^4 CFU/mouse (equivalent to 5400 mg of wet weight/kg) was non-toxic, non-pathogenic and non-infective for mice. A pattern of clearance was established within 7 days after dosing. Note that the test dose was administered based on wet weight; the dry weight was reported to be 18.3% of the wet weight (approximately 988 mg/kg).
Classification: Acceptable.

I. STUDY DESIGN

Test Material: The microbial pesticide control agent (MPCA) is Mycoleptodiscus terrestris mycelia (Lot No. TOX 002). Two preparations of the dosing material were made; one with viable technical grade mycelia (TG) and the other with killed technical grade mycelia (KTG). The test material was prepared by suspending 250mg wet weight of TG or KTG in 1 ml water. The actual dry weight of the test material is reported to be 18.3% of the wet weight.

Test Animals: CD1 mice were obtained from Charles River Laboratories (Portage, MI). They were assigned to the following experimental groups: 36 mice (18/sex) in each of the naive control (NC), the TG and KTG groups, and 12 mice (6/sex) in the shelf control (SC) group. Six of the mice (3/sex) in each group were maintained as 'extras'. Body weights at the beginning of the study were in the approximate range 19-20g for females, and 23-25 g for males.

Methods: All animals were weighed just prior to the beginning of the study (Day 0). Test animals were then dosed, intragastrically, with 0.5 ml of the appropriate test material (TG or KTG) at a concentration of 5.4g of wet weight/kg (approximately 125 mg wet weight /mouse). The numbers of viable fungi administered were determined by plating samples of the dosing suspension on Martin's Agar plates. Body weights were again recorded on Days 3, 7 and 14 and the animals were observed daily for clinical signs of toxicity. Scheduled sacrifices were performed immediately after dosing, and on Days 3, 7, and 14 at which time gross examinations were done, selected organs (lung, brain, kidney, spleen, liver) were removed and weighed, and tissue samples (lung, blood, brain, kidney, liver, spleen, stomach/intestine, feces) were enumerated for viable test material.

II. RESULTS

The numbers of viable fungal mycelia administered to each mouse in the TG group were reported to be approximately 3×10^4 CFU. About 45.7% of this concentration was recovered from the stomach/intestine immediately after dosing (Day 0). Viable organisms were also found in the liver and feces of TG mice at Day 0 but had cleared from the liver and stomach/intestine by Day 3 and from the feces by Day 7. At Day 7, low levels (24 ± 60 to 185 ± 149 CFU/organ) also were found in the stomach/intestine of NC , KTG and SC animals suggesting probable contamination. No data on microbial clearance were given beyond Day 7 because a pattern of clearance was presumed to have been established by this time.

All mice , except one female KTG mouse which died on Day 2, gained weight over the course of the study. There were no statistically significant differences in body weights or weight gains between any group at the conclusion of the study (14 days). There were also

no significant increases in organ weights.

No clinical signs of toxicity were observed except for the unscheduled death mentioned above. Gross necropsies revealed minor abnormalities: a slightly enlarged spleen in one TG mouse on Day 3 and red, mottled lung in one NC mouse on Day 7.

III. SACB DISCUSSION

When mice were administered the technical grade of Mycoleptodiscus terrestris mycelia (100% mycelia) at a concentration of 125 mg/mouse (equivalent to approximately 3×10^4 CFU fungi/Mouse), there was no evidence of toxicity or pathogenicity or persistence. A pattern of clearance was established by Day 7. It is most likely, in SACB's opinion, that the appearance of small numbers of the Mycoleptodiscus terrestris in organs of dosed animals resulted from contamination within the animal facilities or through handling since the fungus also was detected in non-treated and KTG animals.

DATA EVALUATION REPORT (152A-12)

Reviewed by: Cindy Schaffer, Microbiologist, SACB/HED 5
Secondary Reviewer: Rita Briggs, Ph.D., Chemist, SACB/HED 23

Study Type: Acute Pulmonary Toxicity/Pathogenicity - mice
MRID No: 418335-03
Caswell No: 584H
Test Material: Mycoleptodiscus terrestris mycelia
Project No: LO8247
Sponsor: EcoScience Laboratories, Amherst, MA
Testing Facility: IIT Research Institute, Chicago, IL
Title of Report: EPA Subdivision M Tier I Acute Pulmonary Toxicity/ Pathogenicity Testing of Mycoleptodiscus terrestris.
Authors: Robert L. Sherwood
Study Completed: November 1990
Conclusion: This study was designed to test technical grade and killed technical grade material of Mycoleptodiscus terrestris mycelia at a concentration of 200 mg wet weight/kg body weight (approximately 5 mg/mouse). The dose administered is equivalent to 38.4 mg dry weight/kg (approximately 1mg/mouse) and 84 CFU viable fungi/lung. Results from the study showed that 22% (8/36) of TG and KTG mice did not recover from dosing; 16% (5/31) of the remaining TG and 38% (12/31) of the remaining KTG animals died within 3 days after dosing. In a preliminary study, 1/5 mice (20%) died within one day of dosing at the same concentration while the remaining animals had symptoms of toxicity similar to those observed in mice from the TG and KTG groups in the main study. SACB believes that mortalities and toxic effects may have been in response to non-specific toxicity of test material since there was a higher rate of deaths and clinical signs of toxicity in the KTG group. Moreover, no toxic effects were seen after day 9. At the dose administered, Mycoleptodiscus terrestris was non-infective and non-pathogenic; the organism was cleared from the lungs within 48 hours after dosing and no significant necropsy observations other than enlarged spleens in female

mice were seen.

Classification: Acceptable.

I. STUDY DESIGN

Test Material : The microbial pesticide control agent (MPCA) is Mycoleptodiscus terrestris mycelia (Lot No. TOX 001). Two preparations were tested: viable technical grade material (TG) and killed technical grade material (KTG). The test dose was administered based on wet weight (200 mg/kg). Actual dry weight was reported to be 19.2% of wet weight.

Test Animals: CD1 mice were obtained from Charles River Laboratories (Portage, MI). At the initiation of the study, females weighed about 22-24g while males weighed between 25-28g; mice were approximately 6-8 weeks old. Four exposure groups were set up as follows: 36 (18/sex) mice were assigned to each of the naive control (NC), TG treated, and KTG treated groups; five male and six female mice were assigned to the shelf control (SC) group. Six mice (3/sex) in each group were maintained as extras.

Methods: A preliminary study was conducted to determine the highest achievable dose following intratracheal dosing of 2, 20, 200 and 500 mg (wet weight)/kg of TG and KTG in 0.05 ml water . These doses were equivalent to 0.05, 0.5, 5, and 12.5 mg, respectively, per animal; five mice per dose were treated and observed for signs of toxicity over a 5-day period.

A second preliminary study was conducted to evaluate the effect of homogenisation of tissue samples and test materials on the sensitivity of method for detection of the test agent. Serial dilutions of viable fungal (TG) were homogenized with lung or stomach samples removed from uninoculated control animals. Aliquots of homogenized material and untreated TG were plated on Martin's Agar and non-selective media. The numbers of colony forming units were determined and the sensitivity of detection was expressed as percent of recovery.

In the main study, mice were weighed just prior to dosing. Each animal in the treated groups (TG and KTG) was then

administered, via the intratracheal route, 5 mg of test material suspended in 0.05 ml water (equivalent to approximately 200 mg/kg). Viable colony forming units of fungal mycelia delivered were determined by plating serial dilutions of the dosing suspension on Martin's Agar plates. All animals were observed daily for clinical signs of toxicity. Body weights were recorded weekly and at the time of sacrifice. Three mice per sex from each test group were sacrificed immediately after dosing, and on Days 3, 7, 14, and 21. At the time of sacrifice, a gross examination was performed, selected organs (lung, brain, kidney, spleen, liver) were removed and weighed, and samples of organs and tissues (lung, blood, brain, kidney, liver, spleen, and cecum) were enumerated for viable organism.

II. RESULTS

Preliminary Study to determine test dose: It was reported that all animals receiving 5mg, 0.5mg, and 0.05 mg of TG survived dosing. Animals receiving 12.5mg in 0.05 ml had a high mortality rate (3/5 mice died at the time of dosing; one died the following day). One animal receiving the next highest dose (5 mg) died within one day of dosing. The remaining animals in this group had rough hair coats, labored respiration during the 5-day observation period. Results from these studies determined the highest achievable dose to be 5 mg of test material/mouse (equivalent to 0.05 ml of 100 mg wet weight/ml).

Preliminary Study to Determine Sensitivity of Method of Detection: Data on three dose concentrations of TG showed that homogenization of tissue samples and test materials had no significant effect on recovery at the two lowest levels (approximately 400-500 CFU and 8000-9000 CFU). Higher concentrations of fungal mycelia (57,000-66,000 CFU) were recovered less efficiently from lung tissue. Percent recovery from stomach tissue, pre- and post-homogenization, was very high (521 $\pm$ 159 and 344 $\pm$ 285, respectively).

Main Study:

Mortality: When mice were dosed with technical grade (TG) material at a concentration of 0.05 ml of 100 mg wet weight/ml (200 mg/kg or approximately 5 mg/mouse), unscheduled deaths occurred. In response to dosing, 6/18 male* and 2/18 female mice (i.e. 8/36) died. Subsequently, three male mice were replaced and treated with the same dose. By Day 3 after dosing, 2 additional male and 3 female TG mice had died. In summary, 13 out of 39 (33%) mice in the TG group died in response to treatment, the majority (22%) at the time of dosing.

When 18 female and 18 male mice were dosed with heat-killed technical grade (KTG)

material at the same dose (200 mg/kg), unscheduled deaths also occurred. At the time of dosing, 6/18 male* and 2/18 female* TKG mice reportedly died. Three male mice were replaced. By Day 3, additional mice were found dead - 9 male and 3 female. In total, 20 out of 39 KTG mice (approximately 51%) died spontaneously from treatment. Of these, approximately 20% died in response to the dosing.
* Note that there are discrepancies between the number of deaths reported in the text (p.13, Vol. 6) and Tables 4-6 (p.26). The actual number of deaths reported here represent SACB's interpretation of data from both the text and tables.

Body weights: By the termination of the study at day 14, all mice except TG and KTG female mice had gained weight. The latter two groups lost weight until Day 7, but by the end of the study had recovered their initial weights. The difference, when compared to the body weights of the NC group, however, was not statistically significant. Body weights of both male and female mice in the SC group, on the other hand, were significantly lower than those in the NC group by Day 14.

Body weight gains: Final body weight gains for male KTG mice were significantly higher than comparable NC mice. There were no significant differences in weight gains between the other test groups and the NC group.

Clinical signs of toxicity: In addition to mortality, other signs of toxicity observed included rough hair coat, labored respiration, lethargy, hunched posture and tremors. These symptoms were more prominent in the KTG group and during the first 3-4 days after dosing although rough hair coat persisted in the TG group until Day 6 and in the KTG group until Day 9.

Necropsy observations: Acute effects such as red mottled lungs, autolysis, and liver, kidney and lung spots occurred in both treated groups up to 7 days following dosing. Female mice from the TG and KTG groups also had enlarged spleens at day 7. Lesions in kidneys and liver were observed in mice dying spontaneously 2 to 3 days after treatment.

Microbial Clearance: Eighty-three percent of the original inoculum was recovered from the lungs of TG mice at Day 1 and had cleared within 2 days of dosing. The fungi did not appear in any other organ at any time during the test period.

Organ Weights: Treatment with either the TG or KTG material had no significant effect on weights of brain, kidney, spleen, or liver of test animals at any time during the course of the study. The lung weights recorded on Days 3 and 7 appeared to be affected by treatment with either the TG or KTG. However, by the end of the study (Day 14), only

III. SACB DISCUSSION

Intratracheal dosing of 0.05 ml of TG or KTG test material, at a concentration of 5 mg/ml wet weight /mouse (approximately 1 mg dry weight and 84 viable CFU fungi/lung), caused unscheduled mortalities in both groups within the first 3 days. Of these deaths, 22% occurred at the time of dosing in both groups and approximately 16% of deaths in the TG group and 39% in the KTG group occurred within three days after dosing. Other signs of toxicity (rough hair, labored respiration, lethargy, hunched posture and tremors) were seen, predominantly in the KTG group and within the first 3-4 days. Although the registrant attributed the deaths, in part, to the size and quantity of the test article, SACB believes the adverse effects are due to the non-specific toxicity of the test agents because the effects were higher in the KTG animals and the number of viable fungi in the inoculum was very low (84 CFU/lung). Since the test materials were reportedly homogenized, it is unclear to what extent, if any, the size of the TG or KTG contributed to mortalities.

Enlarged spleens were seen only in female TG and KTG mice after 7-14 days. The registrant explained the observation as "a normal immune response to the pulmonary challenge". However, the phenomenon was seen only in females and there were no statistically significant differences in spleen weights reported.

A pattern of clearance from the lungs was clearly established within 48 hours after dosing supporting the non-infectivity of the TG at the dose administered.

DATA EVALUATION REPORT (152A-13)

Reviewed by:	Cindy Schaffer, Microbiologist, SACB/HED
Secondary Reviewer:	Rita Briggs, Ph.D., Chemist, SACB/HED

Study Type:	Acute Intraperitoneal Toxicity/Pathogenicity - mice
MRID No:	418335-04
Caswell No:	584H
Test Material :	Mycoleptodiscus terrestris mycelia
Project No:	LO8247
Sponsor:	EcoScience Laboratories, Amherst, MA
Testing Facility:	IIT Research Institute, Chicago, IL
Title of Report:	Acute Intraperitoneal Toxicity/Pathogenicity Testing of Mycoleptodiscus terrestris, a Fungal Herbicide.
Authors:	Robert L.Sherwood, Ph.D.
Study Completed:	December 1990
Conclusion:	Note that data from two acute intraperitoneal toxicity/ pathogenicity studies were submitted. However, the results from one study (designated as the 'supplemental study' and described in Appendix A), were not considered valid because the test agent was reported to be unstable and no viable organisms could be detected in the dosing suspension or the treated animals. Therefore, SACB based its conclusions on the potential health effects associated with exposure to Mycoleptodiscus terrestris via the intraperitoneal route on data from the second study described in this report. Results from this study indicate that a dose level of 125 mg/mouse (approximately 6.3×10^4 to 3×10^5 CFU of viable or killed Mycoleptodiscus terrestris mycelia) is non-infective, non-pathogenic and non-toxic to mice. Clearance of the organism was completed within 7 days after dosing and this result prompted the study to be terminated at Day 7. Approximately 8% of the TG mice died within 3 days of dosing. Necropsy and clinical symptoms which were present at the termination of the study included enlarged spleens and mesenteric lymph nodes and masses in the peritoneal cavity. SACB believes these symptoms may represent immune defenses and that a 7-

day observation period is too short to fully evaluate recovery from immune challenge. Likewise, the lower body weight gains seen in treated animals would be expected to improve over the normal study observation period of 14-21 days.

Classification: Acceptable.

I. STUDY DESIGN

Test Material: Technical grade (TG) and killed technical grade (KTG) of Mycoleptodiscus terrestris mycelia (Lot TOX 004) is the microbial pesticide control agent in this study. The test material was prepared by suspending 0.25 g wet weight of Mycoleptodiscus terrestris in 1 ml of sterile water and homogenized. The dose administered was 5 g wet weight/kg body weight. Actual dry weight was reported to be 22.3-22.6% of wet weight.

Test Animals: Sixty male and sixty female CD1 mice, obtained from Charles River Laboratories (Portage, MI), were used in the study. At the initiation of the study, the animals were approximately 8 weeks of age and females weighed between 22-25 g, males between 28-30g. Four exposure groups were established: 36 mice (15 male, 15 female and 6 (3/sex) additional mice) were assigned to each of the TG, KTG and NC groups; the SC group comprised a total of 12 animals (3male, 3 female and 6 (3/sex) additional mice to be maintained as extras). The NC and KTG groups were housed together; the SC mice were housed on the same rack as the TG group.

Methods: A preliminary study was conducted to assess the sensitivity of method of detection for the active ingredient following homogenization of stomach and lung samples and test material. Three dose concentrations of test agent were homogenized with previously homogenized tissue samples. Viable fungi were enumerated on Martin's agar plates.

In the main study, mice were weighed just prior to dosing. Each test animal received, via the intraperitoneal route, a single dose of 0.5 ml of the TG or KTG prepared at a concentration of 0.25 g wet weight/ml of water (equivalent to 125,000-590,000 CFU/ml). Body weights were determined weekly thereafter and also on the day of sacrifice or at the time of unscheduled death. Animals also

were observed daily for clinical signs of toxicity. Three mice per sex in each treatment group, except the SC group, were sacrificed immediately following dosing, were washed intraperitoneally with 5 ml of 0.1% peptone and the number of viable organism in the lavage was determined. Six additional mice (3/sex) were sacrificed at Days 3,7, and 14. At the time of sacrifice, or unscheduled death, a gross examination was performed, selected organs and tissues (lung, brain, blood, kidneys, liver, spleen, caecum, mesenteric lymph nodes, lavage) were removed and counted for viable fungi to evaluate infectivity of the organism.

II. RESULTS

Preliminary Study to Determine Sensitivity of Method of Detection: Data on three dose concentrations of TG demonstrate that homogenization of stomach and lung samples and test materials have no significant effect on recovery at the low levels (approximately 400-500 CFU and 8000-9000 CFU). Percent recovery from lung tissue (41 $\pm$31 pre-homogenization vs. 27 $\pm$ 37 post-homogenization), however, was less efficient when greater concentrations of fungal mycelia (57,000-66,000 CFU) were inoculated. Percent recovery from stomach tissue, pre- and post-homogenization, was very high (521 $\pm$ 159 and 344 $\pm$ 285, respectively).

Main Study:

Microbial Clearance: Immediately after dosing (Day 0), Mycoleptodiscus terrestris was enumerated in the peritoneal lavage to determine the actual dose delivered. Of the original inoculum, 50 $\pm$ 57 per cent (89,760 $\pm$ 101,100 CFU) was recovered from the peritoneal cavity . The organism also was found in the kidney, liver, spleen, and cecum at low levels varying between log (1.35 $\pm$ 1.39) to log (3.7 $\pm$ 0.73). However, by Day 3, all organs, except mesenteric lymph nodes and spleen, were cleared and by Day 7 no viable organism was detected in any of the organs. Therefore, complete clearance occurred within 7 days after dosing, and the study was terminated at this time.

Mortality: Unscheduled deaths occurred on days 2-3 when 2 females and 1 male from the TG group (3/36, approximately 8%) were found dead. No deaths were reported from dosing, per se.

Other Clinical Observations: The most common clinical observation among treated animals was rough hair coats. The condition was seen in 37/59 treated mice on Day 3 but by Day 7 only 1 female was observed with rough hair coat.

Body weights/weight gains: All animals gained weight by the end of the study at Day 7. There were no statistically significant differences in body weights between exposure groups although weight gains, in general, were lower in treated groups. Final body weight gains for males in both the TG and KTG groups were significantly lower when compared to corresponding males in the NC group.

Necropsy observations: A gross examination at days 3 and 7 showed that treatment with either TG or KTG induced enlarged spleens and mesenteric lymph nodes as well as masses in the peritoneal and abdominal cavities in all test animals.

Organ weights: Except for liver weights in TG male mice on Day 0, no significant differences were seen in organ weights between exposure groups until Day 7. At Day 7, lung weights of male TG, KTG and SC mice, and weights of mesenteric lymph nodes in SC mice,were significantly higher than corresponding animals in the NC group.

III. SACB DISCUSSION

Although data from two acute intraperitoneal toxicity/pathogenicity studies were provided, only one study was reviewed by SACB. Data from the 'supplemental study' described in Appendix A was considered invalid because the test agent was reported to be unstable and no viable organisms were recovered from either the dosing suspension or the treated animals.

Results from the main study demonstrated that a pattern of clearance was established by Day 7 indicating that Mycoleptodiscus terrestris, as tested under the present conditions, is non-infective. However, 3/36 (approximately 8%) unscheduled deaths in the TG group occurred on Days 2-3 and necropsy revealed enlarged spleens and mesenteric lymph nodes as well as masses in the peritoneal and abdominal cavities in both the TG and KTG groups. Weight gains for the treated male animals, moreover, were lower than the naive controls. However, SACB considers a 7-day observation period too short to fully evaluate the effect of treatment on body weight gains and recovery from immune challenge. The most common clinical symptom among treated animals was rough hair coat. This condition was temporary and SACB considers the test material to be non-toxic.

152A-15: SURVEY OF HYPERSENSITIVITY INCIDENTS

It is reported (Vol. 8) that there are no documented hypersensitivity reactions to the active ingredient formulated Mycoleptodiscus terrestris.

REVIEW OF PROPOSED PROTOCOLS

Antifungal Agent Screening (TGAI)

The protocol submitted (Vol. 1A) is designed to test sensitivity of Mycoleptodiscus terrestris to 5 antifungal agents: nystatin, amphotericin B, miconazole nitrate, griseofulvin, and clotrimazole. SACB agrees with the proposed study.

Determination of the dose at which no intraperitoneal toxicity occurs for toxicity testing of production batches.

Vol. 1B outlines a study to determine the limit for non-specific toxicity (NOEL) in mouse following intraperitoneal injection of Mycoleptodiscus terrestris at five dose levels: 125mg, 62.5 mg, 25 mg, 12.5 mg and 0 mg/mouse/0.5 ml solution. Based on results from the Acute Intraperitoneal Toxicity/Pathogenicity study in mice, SACB does not see the need to test the highest dose and recommends that the response to only two doses (62.5 mg and 31.2 mg) be evaluated.

Storage Stability Studies

This study (Vol. 1C) will determine storage stability of both the formulation and active ingredient at room temperature over a 12-month period. SACB considers the study to be in accordance with Subdivision M, 151A-16 guideline.