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.

5