DATA EVALUATION REPORT (152A-12)

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

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.

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