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DATA EVALUATION REPORT (152A-10)

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STUDY TYPE:	Acute oral toxicity/pathogenic - rat
MRID NO:	416663-04
CASWELL NO:	883AB
TEST MATERIAL:	Metarrhizium anisopliae ESF1
SYNONYMS:	
PROJECT NO:	AFR 902
SPONSOR:	Roussel Bio Corporation, Lincoln Park, N.J.
TESTING FACILITY:	Huntingdon Research Center Ltd., England
TITLE OF REPORT:	Acute oral toxicity and infectivity/ pathogenicity study in rats
AUTHORS:	David J.N. Hossack, Martin Baker, Stuart Denton
STUDY COMPLETED:	August 17, 1990
CONCLUSION:	Metarrhizium anisopliae ESF1 showed no evidence of toxicity or infectivity/ pathogenicity in rats after oral dosing at a concentration of 10^8 CFUs. A pattern of clearance from cecum contents and feces was demonstrated.
CLASSIFICATION:	Supplementary. The study may be upgraded to 'acceptable' when the temperature requirements for the growth of the active ingredient are satisfactorily documented.

I. STUDY DESIGN

Test material: Metarrhizium anisopliae ESF1 (Batch no. PDBAT901) harvested on 3/14/90. The MPCA was prepared by suspending M. anisopliae ESF1 in sterile 2% Tween 80 and phosphate buffered saline (PBS). A volume of 20 ml/kg body weight (containing at least 10^8 CFUs) was administered to each test animal.

Test animals: Fifty Sprague-Dawley origin CR:CD R(SD) BR VAF Plus rats (Charles River U.K. Ltd.) were used in the study. The weights before dosing ranged from 89-138 g. Animals consisted of equal numbers of males and females, and were divided into nine groups. Groups A-E were each assigned 3 male and 3 female rats and were treated with the test material at 20 ml/kg body weight. A control group of 8 rats (Group F; 4 males and 4 females) was treated with the killed organism. Two additional

control groups (Groups G and H) each contained four animals (2/sex) and were not treated with the test substance. Group G served as shelf controls, i.e., were housed in the same room as the treated rats while Group H were housed in a separate room. A final group (Group I) were used as vehicle controls and were dosed with sterile 2% Tween 80 in PBS at 20 ml/kg body weight.

Methods: Food was withheld from all animals overnight prior to dosing. The viable test substance was prepared on the day of dosing (day 1) and administered to the experimental animals in Groups A - E. Group F received the killed organism, Groups G and H were untreated, while animals in Group I were dosed with Tween/PBS.

Individual body weights were recorded on days 1, 2, 4, 8, 15 and 22.

Body temperatures of all rats were recorded on the day before dosing (day 0), on day 1 prior to dosing, and 2, 4, and 24 hours after dosing. An electronic thermometer with an intra-rectal probe was used to measure temperature.

All animals were observed for clinical signs soon after dosing, and at frequent intervals throughout the remainder of day 1. On subsequent days, the animals were observed in the morning and at the end of the experimental day.

Experimental animals were sacrificed by ether inhalation at various times. Group A were killed 24 hours after dosing, Group B on day 4, Group C on day 8, Group D on day 15, and Groups E, F, G, H, and I on day 22. At the time of sacrifice, a gross necropsy was performed. In addition, contents of the stomach, small intestine and cecum were removed for quantitation of the active ingredient.

Fecal and urine samples were collected from Group E in order to assess clearance of the organism. M. anisopliae were counted in 24-hour samples taken on days 2, 3, 4, and 22 using Sabouraud Dextrose Agar plates and selective rice agar salts medium plates.

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II. RESULTS

There were no deaths following treatment with M. anisopliae ESF1. Pilo-erection was the only clinical sign observed and recovery was complete by the end of day 1. No pyrogenic response was noted.

All animals gained weight throughout the study and the group mean bodyweight gains for treated animals remaining at the end of the study were comparable to the control groups.

No differences in mean body temperature were observed between the experimental and control groups.

Gross necropsy did not reveal any abnormalities in tissues. Clearance of the test material was assessed by counting viable spores in the stomach and intestinal contents as well as fecal and urine samples. Data showed that at 24 hours after dosing, small numbers (8×10^2/g) of the organism were recovered from the small intestine of one rat. Thereafter, no viable organisms were isolated from the stomach or small intestine of any rat at any time point. Cecum contents at 24 hours after dosing contained larger numbers of viable spores (range: 6×10^4 - 1.7×10^5/g). At day 3, viable spores (7×10^2/g) were isolated in only 1 of 6 rats. After day 3, the test material was not detected in cecum contents of any rat.

The test material enumerated in fecal contents was reduced during the course of the study. At 24 hours after dosing, a range of 5×10^6 - 1×10^7 viable spores/g were isolated from the feces. At day 3, the numbers were lower (7×10^2 - 7×10^3/g) and no viable spores were recovered 21 days after dosing.

Small numbers of spores were found in the urine samples of 2 of 6 rats on day 1. No viable spores were recovered from any urine sample of any rat thereafter.

III. SACB DISCUSSION

Results from the acute oral toxicity study showed that M. anisopliae ESF1 is neither toxic nor pathogenic to rats. A pattern of clearance from cecum contents and feces was demonstrated. There were no adverse effects on bodyweight gains and no indication of pyrogenic response.

An assessment of infectivity was not included in the acute oral toxicity study on the basis that the temperature requirements (15°-35°C) of M. anisopliae spores would occlude germination at body temperature. However, the registrant assumed that ESF1 spores germinate within the same temperature range as other

M. anisopliae strains and no supporting data or reports were submitted. Therefore, for registration of the test material, SACB would require specific temperature/growth data on the ESF1 strain. Furthermore, if ESF1 spores do germinate at 35°C then this temperature is close enough to body temperature to warrant an evaluation of infectivity.