10-31-91

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

31 OCT 1991

MEMORANDUM

SUBJECT: Review of Toxicology Data in Support of an Experimental User Permit (EUP) Application of the Technical Grade Active Ingredient *Metarrhizium anisopliae* ESF1

TO: Mike Mendelsohn, PM-17
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: John L. Kough, Ph.D., Biologist
Science Analysis and Coordination Branch (SACB)
Health Effects Division (H7509C)

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

DATA REVIEW RECORD

Product Name: *Metarrhizium anisopliae* ESF1
ID no: 000432-EUP-001
Submission no: S398358
Caswell no: 883AB
HED Project no: 1-1585
MRID no: 417149-3 (Acute IV Toxicity);
417149-4 (Acute Pulmonary Toxicity).

ACTION REQUESTED

To review data from acute intravenous and pulmonary toxicology/pathogenicity studies in support of an EUP application for use of fungal technical grade active ingredient *Metarrhizium anisopliae* ESF1.

BACKGROUND

SACB agreed the registrant could perform a more limited number of studies to support the EUP application for use *Metarrhizium anisopliae* ESF1 enclosed in baited cockroach traps. This recommendation was made based on the limited potential for human exposure resulting from this specific non-food application of the active ingredient, the published scientific literature showing *M. anisopliae* to be not a human pathogen and its wide use in Brazilian

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sugar cane not showing adverse health effects.

SACB's CONCLUSIONS

1. SACB continues to support the EUP for this active ingredient as these studies further confirm the finding that Metarrhizium anisopliae ESF1 is not toxic, pathogenic or infective in rats by acute oral, pulmonary or intravenous administration.

2. The fungus has still not been well characterized to confirm it as or distinguish it from other isolates of Metarrhizium anisopliae. Information including a description of the complete fungal life cycle, all known vegetative and resting forms, micrographs of spores and other biochemical and genetic data to identify the isolate should be submitted. Literature references can be used to provide some of this information and more specifics are listed in the memorandum of R. Briggs to P. Bagley/P. Hutton on MRIDs 41666301, 41666303 & 41666304, February 15, 1991.

3. All toxicity and pathogenicity/infectivity studies required for registration of the technical grade active ingredient Metarrhizium anisopliae ESF1 have been submitted. The acute toxicity by the pulmonary and intravenous exposure routes have been reviewed by SACB and are acceptable for registration.

4. The previously reviewed oral toxicity study (MRID 4166604) was classified as supplementary due to the lack of testing the body fluids and major organs for the presence of the test substance. Given the rigorous demonstration of clearance from all the organs by these 2 exposure routes, the oral toxicity study should be upgraded to acceptable.

5. The data base and lack of toxicity and pathogenicity should be adequate to support exemption of the technical grade of M. anisopliae ESF1 from the requirement for a tolerance provided the company submit the taxonomic information requested above.

DISCUSSION

SACB notes that the prior EUP request was for use of the active ingredient M. anisopliae ESF1 in enclosed baited cockroach traps. The Roussel Bio Corporation transmittal document included with this request indicates that this submission is for termite control.

DATA EVALUATION REPORT (152A-13)

Reviewed by: John L. Kough, Ph.D., Biologist, SACB/HED JLK
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SACB/HED RS

STUDY TYPE:	Acute IV Toxicity/Pathogenicity - Rat
MRID NO:	417149-3
CASWELL NO:	883AB
TEST MATERIAL:	Metarrhizium anisopliae ESF1
SYNONYMS:	none
PROJECT NO:	1-1585
SPONSOR:	Roussel Bio Corporation, Lincoln Park, NJ
TESTING FACILITY:	Huntingdon Research Centre Ltd, UK
TITLE OF REPORT:	Acute intravenous toxicity and infectivity/pathogenicity to rats of Metarrhizium anisopliae ESF1
AUTHORS:	David J.N. Hossack, Martin Baker, Stuart Denton
STUDY COMPLETED:	October 25, 1990
CONCLUSION:	Metarrhizium anisopliae ESF1 showed no evidence of toxicity or infectivity/pathogenicity in rats after a single intravenous dose of approximately 2.0 X 10^7 colony forming units (CFU) per animal. The test substance was isolated from all the major organs and fluids except urine examined after dosing. A pattern of clearance was demonstrated from all the major organs examined as well as the blood.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test material: Metarrhizium anisopliae ESF1 (Batch no. PDBAT901) harvested on March 14, 1990. The MPCA was prepared for dosing by suspending M. anisopliae ESF1 in sterile phosphate buffered saline (PBS) with added 2% (v/v) Tween 80. A volume appropriate to deliver a dose of 3.0 ml/kg bodyweight was administered to each test animal (containing approximately 2.00 X 10^7 CFU assuming an average body weight of 122 gm).

Test animal: Fifty-six Sprague-Dawley origin Crl:CD (SD) BR VAF plus rats (Charles River U.K. Ltd.) were employed for the study. The weights before dosing ranged from 110 to 142 gm. The test groups consisted of equal numbers of males and females divided into 10 groups. Groups A-F (3 males and 3 females each) received the test substance dosed at 3.0 ml/kg. The 4 control groups consisted of the following: Group G (4 males/4 females) receiving a dose of autoclaved organisms. Groups H and I (2 males/2 females each) were untreated but were housed in the same area (Group H, ie. a shelf control) or another facility (Group I). Group J (2 animals of each sex) served as the vehicle control and received a dose of sterile 2% Tween 80 in PBS of a volume equivalent to that used for

the treated animals.

<u>Methods</u>: The viable test substance was prepared on the day of dosing (day 1) and injected into the test animals in Groups A-F. Group G received the autoclaved microbes, Group J received a dose of the test vehicle alone (2% Tween 80 in PBS) and Groups H and I were not treated.

Individual body weights were recorded for all surviving animals on days 1, 8, 15 and 22. In addition, the treated animals in Groups B, C and D were weighed prior to sacrifice.

Body temperatures of all rats were recorded the day before dosing (day 0), on day 1 prior to dosing and at 1, 2, 4 and 24 hours after dosing. Temperatures were measured with an electronic thermometer equipped with an intra-rectal probe.

All animals were observed for clinical signs after dosing and frequently for the remainder of day 1. On all 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 the times specified. Group A was sacrificed 1 hour after dosing on day 1, Group B on day 2, Group C on day 4, Group D on day 8, Group E on day 15 and Groups F, G, H, I and J on day 22. Prior to further examination, a blood sample was removed from the orbital sinus. All animals received a gross necropsy at sacrifice. The brain, heart, liver, kidneys, lungs, spleen and mesenteric lymph nodes were removed and sampled for the presence of viable test organism by microbiological culturing on Sabouraud Dextrose Agar. The blood and cecum contents were also cultured by the same methods but employing a selective rice agar salts (SRAS) medium.

Fecal and urine samples were collected from individuals in Group F on day 1/2 and 21/22. During this collection period the test animals received no food. The collected samples were tested for the presence of viable test microbes by culturing on SRAS.

II. RESULTS

There were no deaths following treatment with <u>M. anisopliae</u> ESF1. There were no clinical signs observed throughout the test period. No pyrogenic response was seen in any group.

All animals gained weight throughout the test period and the group mean bodyweight gains for the treated animals were comparable to the control groups. Group F had a lower weight gain than the control groups between day 15 and 22 but this was attributed to the additional days of starvation endured by Group F during urine and feces collection on days 1/2 and 21/22.

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

Gross necropsy showed no visual abnormalities in the appearance of the major organs. Clearance of the test substance was assessed by counting viable colony forming units cultured from the major organs and body fluids. All major organs and the blood had recoverable test substance microbes. The cecum contents and feces had lower and more sporadic recovery of the test microbe. No test substance was recovered from urine samples during the test period. Clearance was demonstrated from all sites where the test substance was detected and no test substance was detected in any experimental animal after the day 14 analysis.

The highest average numbers of viable test microbes were recovered from the spleen on day 4 (an average of 7.35×10^6 CFU/gm). Recovery averages higher than 10^5 CFU/gm were found in the lung, liver and spleen 24 hours after dosing. The highest average levels of test microbe recovery in the other organs ranged from 10^4 (kidneys) to 10^3 (heart, brain, blood and lymph nodes). Test microbe was recovered at less than 200 CFU/gm from the cecum contents of 2 of 6 rats after 1 hour and from 3 of 6 rats after 24 hours. Viable M. anisopliae ESF1 was recovered from the feces of 3 of 6 rats 24 hours after dosing. The highest recovery was 5.08×10^3 CFU/gm feces in 1 female. The cultured test microbe levels dropped consistently from the highest levels for all fluids and organs (except the spleen) until they were no longer detectable in any of the organs or fluids tested. The average levels in the spleen started at 1.23×10^6 CFU/gm and increased until day 4 (7.35×10^6 CFU/gm) then decreased to 1.46×10^4 on day 7 and finally to 26 CFU/gm in 1 of 6 spleens cultured on day 14. No test substance microbes were detected after day 3 in blood and day 7 for all other organs and fluids sampled.

III. SACB DISCUSSION

Results from the acute intravenous toxicity and pathogenicity/infectivity study showed the M. anisopliae ESF1 is neither toxic nor pathogenic to rats. High recoverable fungal populations were initially found in all major organs and blood fluids and declined to lower than the detection limit demonstrating a pattern of clearance. The test substance was also cultured from the cecum contents and feces sporadically at the first test periods. There were no clearly adverse effects on bodyweight gain and no pyrogenic response indicated in the temperature records.

DATA EVALUATION REPORT (152A-12)

Reviewed by: John L. Kough, Ph.D., Biologist, SACB/HED
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SCAB/HED

STUDY TYPE:	Acute Pulmonary Toxicity/Pathogenicity- rat
MRID NO:	417149-4
CASWELL NO:	883AB
TEST MATERIAL:	Metarrhizium anisopliae ESF1
SYNONYMS:	none
PROJECT NO:	1-1585
SPONSOR:	Roussel Bio Corporation, Lincoln Park, NJ
TESTING FACILITY:	Huntingdon Research Centre Ltd., UK
TITLE OF REPORT:	Acute Pulmonary Toxicity and Infectivity/Pathogenicity to Rats of Metarrhizium anisopliae ESF1
AUTHORS:	David J.N. Hossack, Martin Baker, Stuart Denton
STUDY COMPLETED:	October 25, 1990
CONCLUSION:	Metarrhizium anisopliae ESF1 showed no evidence of toxicity or infectivity/pathogenicity in rats after an intratracheal dosing of approximately 1.4 X 10^8 colony forming units (CFU) per animal. A pattern of clearance from the lungs, cecum and feces was demonstrated.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test Material: Metarrhizium anisopliae ESF1 (batch no. PDBAT901) harvested on March 14, 1990. The MPCA was prepared by suspending M. anisopliae ESF1 in sterile 2% (v/v) Tween 80 and phosphate buffered saline (PBS). A volume of this suspension equivalent to delivering 1.2 ml/kg bodyweight (containing at least 10^8 CFU's) was delivered by tracheal instillation to each test animal.

Test Method: Fifty-six Sprague-Dawley origin Crl:CD (SD) BR VAF plus rats (Charles River U.K. Ltd.) were used in the study. The weights before dosing ranged from 245 to 330 gm. for these 7 to 10 week old rats. Animals consisted of equal numbers of male and female rats divided into ten groups. Groups A-F (3 males and 3 females each) were given the test substance by intratracheal inspiration at a dosage of 1.2 ml/kg. Control groups consisted of the following: Group G (8 rats, 4 of each sex) received a similar dose of autoclaved test substance. Group H and I (2 males/2 females each) were not treated but were housed in either the same area (Group H, ie. a shelf control) or in a separate facility (Group I). Group J (2 animals each sex) received a volume of 2% Tween 80 in PBS equivalent to the dose volume.

Methods: The viable test substance was prepared on the day of

dosing (day 1) and administered to the experimental animals in Groups A-F. Group G received a similar dose of autoclaved test substance. Group J received a dose of 2% Tween 80 in PBS. Groups H and I were not treated at all.

Individual body weights were recorded for all the surviving animals on days 1, 8, 15 and 22. Animals in Groups B, C and D were weighed prior to sacrifice on days 2, 4 and 8.

Body temperatures of all rats were recorded on the day prior to dosing (day 0), on day 1 immediately before dosing and 2, 4 and 24 hours after dosing. Temperatures were recorded by electronic thermometer equipped with an intra-rectal probe.

All animals were observed for clinical signs soon after dosing and frequently throughout the first day. On all 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 the times specified. Group A was sacrificed 1 hour after dosing on day 1, Group B on day 2, Group C on day 4, Group D on day 8, Group E on day 15 and Groups F, G, H, I and J on day 22. Prior to further examination, a blood sample was removed from the orbital sinus. All animals received a gross necropsy at sacrifice. The brain, heart, liver, kidneys, lungs, spleen and mesenteric lymph nodes were removed and sampled for the presence of viable test organism by microbiological culturing on Sabouraud Dextrose Agar. The blood and cecum contents were also cultured by the same methods but employing a selective rice agar salts (SRAS) medium.

Fecal and urine samples were collected from individuals in Group F on day 1/2 and 21/22. During the collection periods the test animals received no food. These samples were tested for the presence of viable test microbes by culturing on SRAS.

II. RESULTS

There were no deaths following treatment with M. anisopliae ESF1. The treated animals gained weight over the course of the study at a rate comparable to the controls except for a slight decrease in Groups A and B probably related to anesthesia.

There were several clinical signs noticed in Groups A, B, C, D, E, F, G and J immediately after dosing including piloerection, abnormal gait, lethargy, decreased respiration rate and pallor of the extremities. These symptoms were attributed to the effect of the anesthetic and were not observed after day 5 in any animal.

The mean body temperatures did not differ between the treated and the control animals. No pyrogenic response was seen in the treated or control animals.

Gross macroscopic examination of the organs at necropsy did

not reveal any abnormalities in the tissues. Clearance from the lung and other organs was demonstrated. The test substance was recovered from the lung immediately after dosing at an average recovery of 1.54×10^4 CFU/gm. The test substance average levels in the lung increased after 24 hours (9.47×10^6 CFU/gm) but was found in only 4 of 6 rats. The test substance was thereafter recovered from the lung less frequently and at lower levels until day 21 when none was isolated. This decline started gradually: approximately a fourfold decrease in the 3 animals testing positive at day 7 after dosing. After day 7 there was a 3 log decline in CFU/gm lung tissue in the 3 animals that tested positive. On day 14 there was an average recovery of only 200 CFU/gm lung tissue in 1 animal.

The test substance was also detected in the cecum and the feces. Culturing of the cecal contents of all treated animals tested 24 hour after dosing showed viable test organisms at an average level of 2.48×10^4 CFU/gm. The test of cecal contents on day 4 revealed only 2 of 6 animals testing positive with a tenfold decrease in CFU/gm. The test substance could be recovered from the feces in 3 of 6 animals in Group F 24 hours after dosing. No body fluid or organ except the lungs showed the presence of viable M. anisopliae ESF1 seven days after dosing.

III. SACB DISCUSSION

Results of the study on acute intratracheal dosing of M. anisopliae ESF1 showed it to be neither toxic nor pathogenic or infective to rats with a respiratory exposure. A pattern of lung clearance with high recoverable fungal numbers decreasing over time and appearing in the cecum after the initial pulmonary exposure was demonstrated. These results suggest normal clearance by the muco-ciliary tract with subsequent swallowing and gastro-intestinal passage.

The clinical signs that were noted in all the groups receiving anesthesia disappeared by the fifth day. No adverse effects on weight gain compared to the controls were seen that could not be easily explained by the effect of anesthesia. No pyrogenic response was noted by tracking animal temperatures.