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

16 FEB 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ecological Effects Review of a 154A-16 Tier I Avian oral study of GUS Concentrate Biological Fungicide with *Bacillus subtilis* as Active Ingredient. MRID No. 425783-01. Required as a Conditions of Registration. EPA Reg. No. 7501-144. (Also supports EPA Reg. No. 7501-146)

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Benjamin Chambliss, Acting PM-21 & Carl Grable
Fungicide-Herbicide Branch
Registation Division (H7505C)

The subject microbial pesticide Tier I Avian oral study is of good scientific quality and acceptable. No treatment related mortality or toxicity was observed at a maximum dose of 3,333 mg/kg body weight of GUS 2,000 concentrate of *Bacillus subtilis* as active ingredient administered by gastric lavage to 30 quail for each of five days. The total for five days was 16,665 mg/kg. Neither infectivity nor pathogenicity were reported at necropsy. There were no significant adverse effects reported for weight gain, behavior or feed consumption. Similar absences of mortality, toxicity, pathogenicity and adverse effects were reported for water soluble metabolites and washed spores of *B. subtilis* administered at comparably high dose rates in the study. The test material was considered practically nontoxic and the study is categorized as core.

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EEB Out :

To: Benjamin Chambliss, Acting PM-21 & Carl Grable
Fungicide-Herbicide Branch
Registration Division (H7505C)

16 FEB 1993

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Attached, please find the EEB review of...

Reg./File # : EPA Reg. No. 7501-144
Chemical Name : *Bacillus subtilis*
Type Product : Microbial pesticide
Product Name : GUS 2,000 Concentrate Biological Fungicide
Company Name : Gustafson, Inc., P.O. Box 660065
: Dallas, Texas 75266-0065
Purpose : Review Guidance 154A-16 Tier I Avian oral study

Action Code : 575 Date Due: 02/15/93
: Cond. reg. follow-up data requirement
Reviewer : Robert I. Rose

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GLDN NO	MRID NO	CAT
154A-16	42578301	Y						

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline, but additional information is needed
S=Supplemental (Study provided useful information, but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur
OTHER/COMMENTS/CONCLUSION: There was no mortality at a maximum challenge dose of 3,333 mg/kg body weight of GUS 2,000 concentrate of *B. subtilis* for each of five days. The total for five days was 16,665 mg/kg. Neither infectivity nor pathogenicity were reported. There were also no significant adverse effects reported for weight gain, behavior or feed consumption. Similar results were reported for water soluble concentrates and washed spores of *B. subtilis* given at comparably high dose rates in the study. The test material was considered practically nontoxic in the study and the study was acceptable and categorized as core.

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DATA EVALUATION REPORT

16 FEB 1993

1. Microbial: GUS 2,000 Concentrate Biological Fungicide
Bacillus subtilis, Strain GBO3
Chemical code: 129068
EPA Reg. No. 7501-144 (Also supports EPA Reg. No. 7501-146)

2. Test materials: A: *Bacillus subtilis*, Strain GBO3 (GUS 2,000 Concentrate), a light tan powder, Lot No. 0310-50, Reference # 92361A, assigned Wildlife International (WIL) No. 2221. B: Washed spores of *B. subtilis*, GBO3, (GUS 2,000 Conc.), a light tan powder, Lot No. CLWGLPOO1, Reference # 92361A, WIL No. 2222 and C: Water soluble metabolites of *B. subtilis*, GBO3, (GUS 2,000 conc., water soluble products), orange granules, Lot No. CLWGLPOO1, Reference # 92361A, WIL No. 2223.

3. Study/Action type: Microbial pesticide avian oral toxicity (154A-16)

4. Study identification: *Bacillus subtilis* Stain GBO3: An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite by Susan M. Cambell, Joann B. Beavers & Mark J. Jaber of Wildlife International Ltd., 8598 Commerce Drive, Easton, Maryland 21601, Project No: 301-101, FIFRA Guideline 154A-16. Study completed November 25, 1992 and submitted to Gustafson, Inc., P.O. Box 660065, Dallas, Texas 75266. MRID No. 425783-01.

5. Reviewed by: Robert I. Rose
Biologist
EFED/EEB
Signature:
Date: 02 FEB 1993

Leslie W. Touart
Section 1
Signature:
Date:

6. Conclusion: The study is of good scientific quality, acceptable and valid. No mortality was observed at a maximum dose of 3,333 mg/kg body weight of GUS 2,000 concentrate of *B. subtilis* as active ingredient (A.I.) given by gastric lavage to 30 quail for each of five days. The total for five days was 16,665 mg/kg. Neither infectivity nor pathogenicity was reported at necropsy. There were no significant adverse effects reported for weight gain, behavior or feed consumption. Similar absences of mortality, pathogenicity and adverse results were reported for water soluble metabolites and washed spores of *B. subtilis* given at high dose rates in the study. The study is categorized as core.

8. Background: This microbial pesticide avian oral toxicity study was requested as a condition of registration for use of the product as a seed treatment.

9. Materials and methods:

A. Protocol:
The methods used in conducting the study were based on procedures in Section 154A-16 of the EPA Pesticide Assessment Guidelines, FIFRA Subdivision M, Microbial and Biochemical Pest Control Agents.

B. Test Organism:
Bobwhite quail: *Colinus virginianus* were obtained from Wildlife International, Ltd. production flock, Easton, MD. All birds were from the same hatch on February 24, 1992, pen reared and phenotypically the same as wild types. The birds were acclimated for 20 days until March 16, 1992 when the experiment was begun.

C. Test conditions:
Birds were assigned to treatment and control groups as shown below. Each treatment or control group contained five birds. Additionally, each group contained one undosed, infectivity control bird to assess transmission, for a total of six birds per pen. The birds were too immature to differentiate by sex. Each bird was identified with a wing band.

GROUP	NUMBER OF REPLICATES
I. Negative control	2
II. Infectivity control	12
III. Water soluble metabolites of *B. subtilis*	2
IV. Washed spores of *B. subtilis*	2
V. *B. subtilis* concentrate	6

The pens had floor space that measured about 72 by 90 cm. Ceiling height was approximately 23 cm. The photoperiod was 16 hours of light per day during acclimation and throughout the duration of the study. Average ambient room temperature for this study was 23.2°C ±0.8°C with an average relative humidity of 25% ±10%.

The diet during acclimation and testing was formulated to Wildlife International, Ltd. specifications with corn and soybean meal as main ingredients supplemented with vitamins and minerals, but without antibiotics. The diet contained two ingredients that were not of obvious identity. They were CDP (phosphate source) 0.70% and GL Ferm (Fermatco fermentation by-product source of unidentified growth factors) 0.25%. The chicks were given an additional vitamin supplement in their water from day of hatch until initiation of the test. Water was provided *ad libitum* from the Easton, Maryland public water system.

The following test substance dose rate solutions were prepared fresh daily:

GROUP III: Water soluble metabolites of *B. subtilis* at 667 mg/kg body weight daily for five days. One undosed infectivity control bird was included in each pen.

GROUP IV: Washed spores of *B. subtilis* at 2,766 mg/kg or approximately 8.0×10^{10} spores/kg body weight daily for five days. One undosed infectivity control bird was included in each pen.

GROUP V: *Bacillus subtilis* concentrate at 3,333 mg/kg or approximately 8.0×10^{10} spores/kg of body weight daily for five days. One undosed infectivity control bird was included in each pen.

Pesticide Testing Guidance 154A-16 recommended the infectivity control group should be treated with the microbial pest control agent (MPCA) inactivated in such a way as to retain the structural integrity of the cell. This study differs from the Guidance in that one infectivity control bird was included in each pen of all 12 replicates and neither of the two *B. subtilis* spore treatments were apparently inactivated. In this study, infectivity control birds served as sentinels to assess the potential for infectivity by transmission between treated and untreated birds since the infectivity control birds were not treated.

The treatment consisting of water soluble metabolites of *B. subtilis* is comparable to the control group in the Guidance in which the birds are dosed with sterile filtrate from production cultures.

The study director chose to use a Maximum Hazard Dosage Level approach rather than a range of doses to try to determine LD_{50} effects. Therefore, treatments were administered at the maximum carrying capacity of the water vehicle.

Test substance dosing solutions were prepared fresh daily. The solution containing *B. subtilis* concentrate was dispersed with deionized water to achieve the highest dosable suspension possible. The amounts of test substance used in solutions containing the washed spores of *B. subtilis* and water soluble metabolites of *B. subtilis* were prepared to achieve comparable high dosages. Samples of test substance stock solutions were taken to verify test concentrations administered to birds and sent to Gustafson, Inc. for analyses. All birds dosed were individually weighed and given the appropriate dose suspensions directly into the crop or proventriculus with a stainless steel cannula.

The birds were observed at least twice daily for 30 days in accordance with the Guidance. Individual body weights were

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taken on days 0, 1, 2, 3, and 4 prior to dosing and on days 11, 18, 25 and 30. Average body weight was reported for days 0, 4, 11, 18, 25 and 30 and estimated feed consumption was reported for days 0-4, 5-11, 12-18, 19-25 and 26-30. Feed consumption was presented as an estimate due to waste by the birds.

At termination of the study, all birds were subjected to gross necropsy.

10. Reporting requirements:

A. Mortality/Observable effects criteria:
All birds appeared normal and unaffected by treatment. One incidental mortality occurred in the negative control group. The bird had a leg fracture and was subsequently sacrificed. Clinical observations of the birds given *B. subtilis* showed no signs of pathogenicity. There were also no treatment related effects on body weight or feed consumption.

B. Pathological effects/Gross necropsy:
None of the findings in the treatment or infectivity control groups showed significant evidence of pathogenicity or other treatment related effects. Incidental findings were a pale spleen in the bird sacrificed because of a broken leg, a slight dorsal head curl on day 30 in one of the *B. subtilis* treated birds, one male in the negative control group with feather loss on the legs, one female given the water soluble metabolites of *B. subtilis* with a pasty vent, one bird treated with the *B. subtilis* concentrate with a slightly hemmoraghic spleen and another with feather loss on the head. These incidental observations are within the scope of normal variations with this species held in captivity and handled regularly. However, the study report was deficient by omission of the raw data containing the incidental observations as well as individual body weight raw data.

C. Calculated LD_{50}:
Absence of a range of mortality obviated the determination of an LD_{50}. The test used a Maximum Hazard Dose Level approach rather than a range of doses to determine an LD_{50}. Treatments were administered at the maximum carrying capacity of the water vehicle. Thirty birds were treated with 3,333 mg/kg daily for five days. The total for all five days was 16,665 mg/kg. This exceeded the 71-1 avian acute oral requirement being that in lieu of a calculated LD_{50}, the study may demonstrate that the LD_{50} is greater than 2,000 mg/kg.

D. Body weight and food consumption:
No apparent treatment related effects were observed on body weight or feed consumption in any of the treatment groups.

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E. Acceptable protocols:
The deviations from recommended procedures in this study are not of significant.

11. Good Laboratory Practice Quality Assurance:

The following GLP statement was signed by Susan M. Campbell, Senior Research Biologist and Mark Jaber, Laboratory Management of Wildlife International, Ltd.:

"This study was conducted so as to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989."

12. Reviewer's conclusions:

A. Test procedure: The protocol used was substantially similar to the 154A-16 Guidance.

B. Statistical analysis: Not necessary because of no mortality.

C. Discussion:
This study demonstrates lack of oral toxicity of GUS 2,000 concentrate with the A.I. being spores of *B. subtilis* administered by gastric lavage at a maximum dose rate of 3,333 mg/kg of body weight for each day of five days (total of 16,665 mg/kg) to bobwhite quail. Lack of toxicity was similarly demonstrated for high dose rares of water soluble metabolites and washed spores of *B. subtilis*. No significant pathological or infectivity effects were observed and neither were adverse effects reported for weight gain, behavior or feed consumption.

The Application for Pesticide "Other", EPA Form 8570-1, misdated December 1, 1993 submitted with this study states under Explanation: "Also required by Notice of Pesticide Registration Dated 7/9/92 for Kodiak HB, EPA Reg. No. 7501-145." This EPA Reg. No. is for a product named Prevail Apron Terrachlor Vitavax Fungicide that does not contain *B. subtilis* as A.I. The EPA Reg. No. for the Kodiak product is 7501-146.

D. Adequacy of study:
Category: Core