3-7-94

EPA, OPP, EFED BIOLOGICALS REVIEW

TO: Sidney Jackson, Acting PM-21, Julie Fairfax & Carl Grable
Fungicide-Herbicide Branch
Registration Division (H7505C)

BIOCHEMICAL/MICROBIAL AGENT: Gustafson, Inc. *Bacillus subtilis*
DP BARCODE: D194497

TEST MATERIAL: *B. subtilis*, MBI 600 (GUS 376 & 378 technical concentrates for nontarget plant and avian oral studies, respectively)

ACTION/STUDY TYPE: 130 NEW BIOL-FOOD/FEED USE

STUDY IDENTIFICATION: MRID # 42682901, An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite **and** MRID # 41907408, Evaluation of Bacillus subtilis MBI600 (ATCC No. SD1414) for Its Effect on Soybean (*Glycine max*)

REVIEWED BY:

Robert I. Rose, Ph.D.
Environmental Fate &
Effects Division (H7507C)

Signature: [signature]
Date: 01/MAR/094

PEER REVIEW BY:

Robert W. Pilsucki, Ph.D.
Environmental Fate &
Effects Division (H7507C)

Signature: [signature]
Date: 3/2/94

BRANCH APPROVAL:

Anthony F. Maciorowski, Ph.D
Chief, Ecological Effects Branch
Environmental Fate &
Effects Division (H7507C)

Signature: [signature]
Date: 3/7/94

CONCLUSION: This review concludes that registration of Gus 376 Concentrate Biological Fungicide would have minimal effects on nontarget organisms.

RECOMMENDATIONS: This review finds no significant objection to the EPA registration of Gus 376 Concentrate Biological Fungicide.

BACKGROUND: Gustafson, Inc. has three products presently registered based on *B. subtilis* as active ingredient (AI). They are EPA registration numbers 7501-144, -146 and -148. The subject 7501-RUT registration application is for *B. subtilis* strain 376. According to the applicant, "the spectrum of diseases controlled by both strains are substantially the same as

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determined by our laboratory tests. The chief differences between the strains are that Gus 376 is able to more rapidly colonize root systems on certain crops and in colonial morphology. No other significant differences have been noted for the two strains." Additionally, *Bacillus subtilis* is not normally considered pathogenic.

The September 12, 1991 Agency letter to the applicant stated: "Although a waiver was requested for avian testing, the Agency feels that the submission of an Avian Oral Pathogenicity/ Toxicity Test (154A-16) using Bobwhite Quail will be necessary in order to assess potential risk to nontarget birds due to ingestion of the treated seeds. The suggested use of a label statement stating that the applicator should cover or incorporate spilled seed or seed on soil surface is not sufficient to alleviate this concern." The EEB memorandum of August 20, 1991 stated: "All of the data requirements have been satisfactorily addressed except for the avian testing." The applicant had requested waivers of all microbial pesticide nontarget organism data requirements under CFR 40 § 158.740.

DISCUSSION OF INDIVIDUAL STUDIES:

1. MRID # 42682901, An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite: The study was conducted adequately. The test procedures adequately followed those of Section 154-16 of Subdivision M of the Pesticide Testing Guidelines, Microbial and Biochemical Pest Control Agents. However, one avian species was tested instead of two as specified in the Guidance. The study demonstrated that neither *B. subtilis*, MBI-600 Technical Concentrate, water soluble metabolites of *B. subtilis*, MBI 600 and washed spores of *B. subtilis*, MBI 600 were toxic or pathogenic to bobwhite quail. The test material was identified as *B. subtilis* strain Gus 378 while the application is for Gus 376. The category of the study was core.

2. MRID # 41907408, Evaluation of Bacillus subtilis MBI600 (ATCC No. SD1414) for Its Effect on Soybean (*Glycine max*): The study was conducted adequately. The references provided in the study that implicated *B. subtilis* as the causal agent for Bacillus seed decay on soybean were all from one laboratory. The laboratory no longer has the *B. subtilis* cultures referred to in the original 1977 publications and a representative of the laboratory has indicated that the identity of the cultures involved was probably *B. megaterium* rather than *B. subtilis*. This study demonstrated that GUS 376 Technical Concentrate lot 0347-51 of *Bacillus subtilis* MBI600, strain Gus 376, (ATCC No. SD-1414) is nonpathogenic to Asgrow cultivar A3427 of soybeans, *Glycine max*. The category of the study was core.

COMPLETION OF ONE-LINER: None
CBI APPENDIX: Confidential Statment of Formula, EPA Form 8570-4

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3-2-94

DATA EVALUATION RECORD

MRID # & TITLE OF STUDY: MRID # 42682901, An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite

REVIEWED BY:

Robert I. Rose, Ph.D.
Environmental Fate &
Effects Division (H7507C)

Signature:

Date: 01 MAR 1994

PEER REVIEW BY:

Robert W. Pilsucki, Ph.D.
Environmental Fate &
Effects Division (H7507C)

Signature:

Date: 3/2/94

REVIEW CONCLUSIONS: The study adequately demonstrated that neither *B. subtilis*, MBI-600 Technical Concentrate, water soluble metabolites of *B. subtilis*, MBI 600 or washed spores of *B. subtilis*, MBI 600 were toxic or pathogenic to bobwhite quail.

ADEQUACY OF STUDY: Core
RECOMMENDATIONS: None
MATERIALS & METHODS: Adequate. There were two replicates of five birds for each of three treatments. The control groups consisted of both a negative control and an infectivity control. The infectivity control consisted of a sixth bird placed in each pen in the treatment and negative control groups. Infectivity control birds served as sentinels to assess the potential for spread of infection.

REPORTED RESULTS: There were no mortalities in the negative control and infectivity control groups. All birds were normal in appearance and behavior throughout the study period. There were no moralities or overt signs of toxicity among birds administered *B. subtilis*, MBI 600 (GUS 378 Concentrate), washed spores of *B. subtilis*, MBI 600 or the water soluble metabolites of *B. subtilis*, MBI 600. Clinical observations of the birds administered *B. subtilis*, MBI 600 gave no indication of pathogenicity. When compared to the negative control and infectivity control group, there were no apparent treatment related effects upon body weight or feed consumption in any of the treatment groups. At termination of the study, all birds were subjected to gross necropsy. None of the findings in the treatment or infectivity control groups indicated evidence of pathogenicity or other treatment related effects.

DISCUSSION: The study was adequately conducted. The test procedures followed those of Section 154-16 of Subdivision M of the Pesticide Testing Guidelines, Microbial and Biochemical Pest Control Agents. However, one avian species was tested instead of two as specified in the Guidance.

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DATA EVALUATION RECORD

MRID # & TITLE OF STUDY: MRID # 41907408, Evaluation of *Bacillus subtilis* MBI600 (ATCC No. SD1414) for Its Effect on Soybean (*Glycine max*)

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Environmental Fate &
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PEER REVIEW BY:

Robert W. Pilsucki, Ph.D.
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REVIEW CONCLUSIONS: This study adequately demonstrates that Gus 376 Technical Concentrate lot 0347-51 of *Bacillus subtilis*, MBI600 (ATCC No. SD-1414) is nonpathogenic to Asgrow cultivar A3427 of soybeans, *Glycine max*.

ADEQUACY OF STUDY: Core

RECOMMENDATIONS: None

MATERIALS & METHODS: Adequate, however, raw data was not included with the study

REPORTED RESULTS: The results of the study demonstrated no effect of seed treatment with *B. subtilis* on normal seedlings produced after 8 days. There was an effect of temperature with no normal seedlings produced by any treatments at 40°C. *Bacillus subtilis*, MBI600 (ATCC No. SD-1414) is not a soybean seed decay organism as measured by the conditions of this test.

DISCUSSION: The study was adequately conducted. The references provided in the study that implicated *B. subtilis* as the causal agent for Bacillus seed decay on soybean were all from one laboratory. The laboratory no longer has the *B. subtilis* cultures referred to in the original 1977 publications and a representative of the laboratory indicated that the identity of the cultures involved was probably *B. megaterium* rather than *B. subtilis*.

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