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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

July
~~JUN~~ 15 2005

MEMORANDUM

SUBJECT: BPPD Review of Storage Stability Data to Support EPA Reg. No. Former 7501-191 (Gustafson LLC), Present 264-985 Submitted by Bayer, which contains *Bacillus pumilus* GB34 (Data Package No.: 311686; MRID No.: 46379701).

TO: Anne Ball (PM-90), Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511C)

FROM: Carl Etsitty, M.S., Microbiologist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511C)

THROUGH: John L. Kough, PH.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511C)

ACTION REQUESTED: Review of the submitted data for storage stability to determine the adequacy of data to support File Numbers 264-985, containing *Bacillus pumilus* GB34, as the active ingredient.

~~THIS REVIEW CONTAINS CONFIDENTIAL BUSINESS INFORMATION~~

BACKGROUND: In the initial submission, Encore Technologies, LLC. memorandum ... Environmental Protection Agency, Subject GB34, File Numbers 7501-ROE & 7501-ROR Amendment to the Manufacturing Process dated October 31, 2002. Encore Technologies, LLC. requested the manufacturing process to be kept confidential from the applicant. Gustafson LLC ... response to additional data or justification was needed to support Gustafson LLC ... to support ... application File Numbers 7501-ROE and 7501-ROR which contains *Bacillus pumilus* GB34 ... active ingredient (Memo dated October 15, 2002, Etsitty to Ball).

CONCLUSION: The submission packet contains adequate storage stability data for the GB34 Technical product. There were minor deviations to the protocol, but enough to impact the study. The supplemental data has been requested, and subjected to SUPPLEMENTARY status pending resolution of what media is used on each of the Certificate of Analysis report.

DATA REVIEW RECORD

Active Ingredient: *Bacillus pumilus* (GB34 Technical)
Product Name: GB34 Concentrate
Company Name: Gustafson Seed Technology Center
ID No: 07501-191
Chemical No.: 006493
Submission No.: S606328
DP Barcode: D279249
MRID No: 461033-01 Storage Stability (OPPTS 830.6317)

SUMMARY OF DATA SUBMITTED:

463797-01: The Corrosion Characteristics and Storage Stability of GB 34 Concentrate End Use Product.

> GB 34 Concentrate containing *Bacillus pumilus* GB 34 stored under ambient conditions either in glass or fiber wood container, was inoculated onto artificial growth media at 5 time periods (study initiation, 3, 6, 12, 24 and 36 mon). No consistent downward growth trends were observed over the 3-year period. The test substance appeared to be stable.

CLASSIFICATION: Supplemental but up gradable to Acceptable with a clarification what media is utilized to observed CFUs, on Certificate of Analysis.

Study Type:	Storage Stability (OPPTS 830.6317)
Test Material:	GB 34 Concentrate
Project No:	PC/00-005SS
Sponsor:	Gustafson LLC, Plano, TX
Testing Facility:	Gustafson R&D Center, McKinney, TX
Title of Report:	The Corrosion Characteristics and Storage Stability of GB 34 Concentrate End Use Product (OPPTS 830.6317)
Author(s):	Pat McFadden Bates
Study Completed:	September 27, 2004
Good Lab. Practice:	Compliant with deviation described.
Classification:	Acceptable

MRID No:	463797-01	The Corrosion Characteristics and Storage Stability of GB 34 Concentrate End Use Product.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: GB 34 Concentrate strain of dried *Bacillus pumilis* (GB34 technical)

Description: Beige to off-white free-flowing powder
Source: Gustafson Seed Technology Center, McKinney, TX
Lot Nos. : PC/00-005M
Composition (Purity): not provided in report
Date of Production not provided in the report

2. Growth Media /Source:
Tryptic Soy Agar, or Potato Dextrose Agar, Difo, for bacteria – SOP# 6.22 Enumeration of Microorganisms by Dilution Plate Counts;
or Potato Dextrose Agar, Difo, for fungi – SOP# 6.22

B STUDY DESIGN AND METHODS

The study is being carried out over a 3-year period. During that time, the test substance is being tested for stability by determining bacterial colony at six time points: at the study initiation, and following storage under ambient conditions (i.e., glass and fiber board container) for 3, 6, 12, 24 and 36 months.

The media sterilized as according to SOP 6.22 Preparation of Plating Medium. The contents of the container (test substance) were weighed or measured by volume either to 0.1 ± 0.02 g for powders, 0.1 ± 0.05 mL for liquids into 16 x 125 mm dilution bottles containing either 9.9 mL ± 0.05 mL or 9.0 ml ± 0.05 mL saline water. After appropriate amount of sample added, the solution is mixed for 30 sec, or until homogeneous. It can be further diluted to 10^{-6}.

Colonies were counted from appropriate serial dilutions and mean titers determined based on

plates. The microbial titers were expressed as colony forming units per gram of test substance (cfu/g).

II RESULTS AND DISCUSSION:

A. TEST MEDIA GROWTH

Viability of GB 34 Concentrate on Tryptic Soy Agar and Potato Dextrose Agar throughout the 3-year test interval ranged from 1.2×10^9 cfu/g to 9.9×10^9 cfu/g for glass container; and, 1.4×10^9 cfu/g to 7.5×10^9 cfu/g in fiber board container.

No statistical analyses were performed; however, overall, the test substance appears to be stable under ambient conditions. Titers fluctuated over the 3-year period but no apparent log reduction on the medium.

Table 1. Stability Data for GB 34 Concentrate
(from Tables 1 of the report)

Time	Analysis Date	Glass Container	Fiber Board Container
0 mon	08/22/00	7.2×10^9 cfu/g	NA
3 mon	12/06/00	1.9×10^9 cfu/g	3.3×10^9 cfu/g
6 mon	02/26/01	2.9×10^9 cfu/g	1.6×10^9 cfu/g
12 mon	08/22/01	2.4×10^9 cfu/g	1.4×10^9 cfu/g
24 mon	08/19/02	9.9×10^9 cfu/g	7.5×10^9 cfu/g
36 mon	10/06/03	1.2×10^9 cfu/g	7.5×10^9 cfu/g

*Samples analyzed according to Gustafson SOP 6.22

13544

R147610

Chemical:	**Bacillus pumilus GB34**
	PC Code: 006493
HED File Code:	**41500 BPPD Tox/Chem**
Memo Date:	**7/15/2005**
File ID:	**DPD279249**
Accession #:	**000-00-9002**

HED Records Reference Center
6/28/2007