UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Review of avian toxicity/pathogenicity study for Gustafson, LLC Experimental Use Permit (PC Code No. 007501-EUP-G; MRID No. 452940-05).

FROM: Zig Vaituzis, Microbiologist
Biopesticides and Pollution Prevention Division, 7511C

PEER REVIEW: Gail Tomimatsu, Plant Pathologist 6/13/01
Biopesticides and Pollution Prevention Division, 7511C

Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division, 7511C

TO: Anne Ball, Regulatory Action Leader
Biopesticides and Pollution Prevention Division, 7511C

CLASSIFICATION: The submitted study "GB-34: An Avian Oral Pathogenicity and Toxicity Study in the Northern Bobwhite" is classified as **Acceptable**, it is scientifically sound and fulfils the OPPTS Guideline 885.4050 testing requirement for avian hazard assessment.

STUDY RESULTS: No overt signs of illness or abnormal behavior were observed following administration of 3.4×10^{11} cfu/kg of body weight of *Bacillus pumilus* GB-34 by oral gavage per day for 5 days followed by a 25 day observation period. No effect on mean body weight was seen. No toxicity, pathogenicity and no treatment-related mortality were observed.

BPPD CONCLUSIONS: The results of the study indicate that no avian hazard is expected from the proposed uses of *B. pumilus* GB-34. *B. pumilus* is one of the most numerous *Bacillus sp.* found on plant surfaces. This study confirms the contention that *B. pumilus* will not pose a hazard to avian wildlife at use rates that may exceed the level naturally occurring on plants.

DATA EVALUATION RECORD
'AVIAN ORAL, TIER I

1. **CHEMICAL:** *Bacillus pumilus* GB-34 PC Code No.: 007501-EUP-G

2. **TEST MATERIAL:** GB-34 Technical, Lot No. H100:95-1 Purity: 0.28%

3. **CITATION:**

Authors:	Gallagher, S.P. and J.B. Beavers
Title:	GB-34: An Avian Oral Pathogenicity and Toxicity Study in the Northern Bobwhite
Study Completion Date:	September 20, 2000
Laboratory:	Wildlife International, Ltd.
Laboratory Report ID:	301-103
Sponsor:	Gustafson, LLC
MRID No.:	452940-05
DP Barcode:	D273326/007501-EUP-G

4. **REVIEWER**[1]: Zig Vaituzis, Ph.D. 6/12/01

5. **PEER REVIEW :** Gail Tomimatsu, Ph.D. 6/13/01

6. **STUDY PARAMETERS**:

Scientific Name of Test Organism: *Colinus virginianus*
Age of Test Organisms at Test Initiation: 21 days
Definitive Study Duration: 30 days

7. **CONCLUSIONS:** No overt signs of illness or abnormal behavior were observed following administration of 3.4×10^{11} cfu/kg of body weight of *Bacillus pumilus* GB-34 by oral gavage per day for 5 days followed by a 25 day observation period. No effect on mean body weight was seen. No toxicity, pathogenicity and no treatment-related mortality were observed.

Results Synopsis:

LC_{50}: $>1.7 \times 10^{12}$ cfu/kg
NOEL: $>1.7 \times 10^{12}$ cfu/kg
Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: Acceptable (See 14. Reviewer's Comments)

[1] Parts of the review were performed by Patricia H. Reno, M.S.

B. Rationale: Methods used in conducting study were based on USEPA OPPTS Microbial Pesticide Test Guideline 885.4050

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: None. (See 14. Reviewer's Comments)

10. SUBMISSION PURPOSE:

To evaluate the acute toxicity and pathogenicity of GB-34 when administered orally to northern bobwhite.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *An upland game bird species, preferably the bobwhite (Colinus virginianus).*	*Colinus virginianus*
Age at beginning of test: 10-14 days old.	21 days, immature – could not be differentiated by sex
Supplier	Wildlife International, Ltd Production Stock
Chicks appeared healthy and did not have excessive mortality before the test?	Chicks were healthy, all birds were from the same hatch, pen-reared, and phenotypically indistinguishable from wild birds
Acclimation period: As long as possible.	21 days

B. Test System

Guideline Criteria	Reported Information
Pen size: about 35 × 100 x 24 cm	72 × 90 ×23 cm, constructed of galvanized wire mesh and sheeting (Beacon Steel Products Model No. B735Q)
Brooder temperature: about 35EC (95EF)	38°C until 21 days old
Room temperature: 22-27EC (71-81EF)	25.5 ± 0.9°C
Relative humidity: 30-80%	65 ± 13%
Adequate ventilation?	Not specified; housing practices based on NRC guidelines
Photoperiod Minimum of 14 h of light.	16L8D, 132 lux

Guideline Criteria	Reported Information
Diet: A commercial gamebird diet.	Gamebird ration formulated to WI specifications, Vitamin supplement in water until test initiation. Water (Easton Public Water Supply) and feed provided *ad libitum* during initiation and testing. No antibiotics were used.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported
Definitive Test **Nominal concentrations:** Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm.	One treatment group receiving oral dose @ 3.4×10^{11} cfu/kg of body weight/day for 5 days. Total dose – approximately 1.7×10^{12} cfu/kg bw.
Controls: Control group tested with diet containing the maximum amount of vehicle used in treated diets?	Negative Control with reverse osmosis water Attenuated Control with autoclave inactivated GB-34
Number of birds per group: 10 (strongly recommended)	Treatment group – 30 (6 replicates of 5 birds each) Control groups – 10 (2 replicates of 5 birds each)
Vehicle: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	Reverse osmosis water
Vehicle amount (% of diet by weight): Not more than 2%	Total volume of dosing solution = 10 ml/kg of body weight/day for five days
***Test durations*:** 5 days with treated feed and at least 3 days observation with "clean" feed	Acclimation – 21 days; Oral Dosing – 5 days; Post-dosing Observation – 25 days
No mortality during last 72 hr of observations?	No *treatment-related or control mortality*. Single incidental mortality (subcutaneous hematoma on head and neck) in treatment group on day 16 with no signs of toxicity; behavior and appearance normal prior to death

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes, QA – page 4: GLP – page 3
Body weights measured at beginning and end of study?	Measured Days 0,1,2,3,4 during dosing; Days 11, 18, 25, and 30 during post-dosing observation
Estimated consumption per pen reported for pretreatment, treatment, and observation periods?	Average estimated feed consumption measured on Days 0-4, 5-11, 12-18, 19-25, and 26-30.
Control Mortality: Not more than 10%	No mortality in negative control or attenuated control

'AVIAN ORAL. TIER I MRID: 452940-05

Guideline Criteria	Reported Information
Raw data included?	Yes, Tables 1 – 3, pages 16-19; Appendices V and VI
Signs of toxicity (if any) were described?	Necropsy results in control groups were not remarkable. In treatment group, feather loss and bruising on head of one bird; abrasions on head of one bird; retained yolk sac in abdominal cavity of one bird. Necropsy for all other birds in treatment group not remarkable. No effects on mean body weight of birds in attenuated control group and treatment group when compared to negative control. Bioassay of test and attenuated materials showed viability to be in an acceptable range.

Mortality:

Nominal Conc. (ppm)	No. of Birds	Cumulative Number of Dead					
		Day of Study					
		1-5	6-10	11-15	16-20	21-25	26-30
Negative control	10	0	0	0	0	0	0
Attenuated control	10	0	0	0	0	0	0
3.4×10^{11} cfu/kg BW/day	30	0	0	0	1[a]	0	0

[a]Mortality not treatment-related

Other Significant Results:

No-observed-effect-dose: 3.4×10^{11} cfu/kg BW/day for 5 days

Statistical Results

Statistical Method: SD on weight and feed consumption data

LC_{50}: >1.7 x 10^{12} cfu/kg

NOEL: >1.7 x 10^{12} cfu/kg

Probit Slope: N/A

13. **VERIFICATION OF STATISTICAL RESULTS**: N/A

14. REVIEWER'S COMMENTS:

The OPPTS Guideline allows for a single group of birds (at least 30) to be tested at the maximum hazard dose, determined using the following formula:

MPCA in TGAI x 5 mL/kg BW x weight of test bird (kg).

The test dose was 10 mL/kg body weight daily for 5 days, for a total of 1.7×10^{12} cfu/kg of body weight administered to 30 birds in 6 replicates. The dosage calculations were not provided. However, since no effect on mean body weight, mortality, toxicity, pathogenicity or abnormality during gross necropsy was observed at the test dose, the study is considered adequate. In addition, it is noted that OPPTS Guideline 885.4050 recommends testing two species of birds to take into account differences in gastrointestinal physiology. However, when the active ingredient is not related to any microorganism that is known to affect birds (as is the case with *B. pumilus* GB-34), testing of only one species is sufficient.

The results of the study indicate that no avian hazard is expected from the commercial uses of *B. pumilus* GB-34.

DP BARCODE: D273326

CASE: 066998 DATA PACKA 03/09/01
SUBMISSION: S593747 BEAN S 1 of 1

* * * CASE/SUBMISSIO

CASE TYPE: EUP (SECT 5) ACTION:
CHEMICALS: 006493 Bacillus pumilus GB 0.2800%

ID#: 007501-EUP-G
COMPANY: GUSTAFSON LLC
PRODUCT MANAGER: 90 JANET ANDERSEN CS1 5TH FL
PM TEAM REVIEWER: ANNE BALL CS1 5TH FL
RECEIVED DATE: 12/27/00 DUE OUT D...

* * * DATA PACKAGE INFORMATION * *

DP BARCODE: 273326 EXPEDITE: Y DATE SENT: 03/09/01 DATE RET.: / /
CHEMICAL: 006493 Bacillus pumilus GB34
DP TYPE: 001
CSF: Y LABEL: Y

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 05/28/01
DIV : BPPD	/ /	/ /	NEGOT DATE: / /
BRAN: BPPD-IO	/ /	/ /	PROJ DATE: / /
SECT: IO	/ /	/ /	
REVR :	/ /	/ /	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

To John Kough: Please review the studies for N-F EUP for B pumilus GB34 for following MRIDS: 452940-01, Acute Oral Toxicity; 452940-02, Primary Eye Irritation; 452940-03 Primary skin irritation; 453416-01, Toxicity/Pathogenicity following IV challenge; 452940-04, The physical characteristics of end use product; 453416-02, The physical characteristics of GB34 Technoical Product; 452940-05, An avian oral pathogenicity and toxicity study in the Northern Bobwhite; 452940-06, Identification of GB34, Bacillus pumilis; 452940-07, Determination of residues of GB34 in processed fractions of soybeans.

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

13544

R144509

Chemical: Bacillus pumilus GB34

PC Code:
006493

HED File Code: 41300 BPPD Eco Effects
Memo Date: 6/13/2001
File ID: DPD273326
Accession #: 000-00-9002

HED Records Reference Center
6/28/2007