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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

April 11, 2002

Memorandum

Subject: BPPD Review of Acute Oral Toxicity Study in Rats--Limit Test; Primary Eye Irritation Study in Rabbits; and Primary Skin Irritation Study in Rabbits Submitted by Gustafson LLC to Support Registration of GB 34 Technical [Submission# S606318; ID. # 7501-ROE; DP Barcode# D279234; Chemical# 006493].

To: Anne Ball, Regulatory Action Leader
Microbial Pesticide Branch,
Biopesticides and Pollution Prevention Division (7511C)

From: Susanne Cerrelli, Biologist 4/11/2002
Microbial Pesticide Branch,
Biopesticides and Pollution Prevention Division (7511C)

Thru: Ibrahim S. Barsoum, Ph.D., Microbiologist Ibrahim S. Barsoum 4/11/02
Microbial Pesticide Branch,
Biopesticides and Pollution Prevention Division (7511C)

ACTION REQUESTED: To review acute oral toxicity study in rats--limit test; primary eye irritation study in rabbits; and primary skin irritation study in rabbits submitted by Gustafson LLC to determine if it is adequate to support registration of GB 34 Technical.

~~THIS REVIEW CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION~~

DATA REVIEW RECORD

Active Ingredient: *Bacillus pumilus* strain GB34
Product Name: GB 34 Technical
Company Name: Gustafson LLC
ID No: 7501-ROE
Chemical Number: 006493
Submission Number: S606318
DP Barcode: D279234D
MRID No:

45433501 Moore, G. (2001) Acute Oral Toxicity Study in Rats--Limit Test: GB 34 Technical: Lab Project Number: 10085: P320. Unpublished study prepared by Product Safety Labs. 14 p. {OPPTS 870.1100}

45433502 Moore, G. (2001) Primary Eye Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10086: P324. Unpublished study prepared by Product Safety Labs. 16 p. {OPPTS 870.2400}

45433503 Moore, G. (2001) Primary Skin Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10087: P326. Unpublished study prepared by Product Safety Labs. 15 p. {OPPTS 870.2500}

BACKGROUND:

B. pumilus is a naturally occurring soil microorganism that act as an antifungal agent. A section 3 registration was requested for the manufacturing use product EPA Reg. File No. 7501-ROE, GB34 Technical Biological Fungicide. Data reviews concerning a related pending experimental use permit, 7501-EUP-G, labeled for seed treatments were recently completed in BPPD. *Bacillus pumilus* strain GB34 did not appear to be toxic, infective or pathogenic in rats that were treated in the acute injection toxicity/pathogenicity study (MRID 453416-01). Product chemistry deficiencies were noted in the manufacturing process data review dated February 4, 2002 and the product chemistry review dated December 21, 2001, that are required to be addressed for the GB34 Technical, including :(a) an additional 4 batch analysis including a discussion of enforcement method for batches that do not meet QA/QC for AI amounts or microbial contaminants. (b) an analysis of microbiological purity was not performed, and (c) packaging information. GB 34 Technical contains the active ingredient *Bacillus pumilus* strain GB34. GB34 Technical Biological Fungicide is labeled for reformulating into registered end-use products. Although prior communications regarding the Experimental Use permit for 7501-EUP-G indicate that the GB34 Concentrate will be applied to soybeans as a seed treatment at EUP sites, complete information are needed concerning the proposed use sites, application methods, and rates of application for section 3 registration of the new active ingredient . A preliminary screening of all the submitted toxicological and product chemistry data to perform a risk assessment for *Bacillus pumilus* strain GB34 revealed several data gaps that have not been adequately addressed.

DISCUSSION:

Most of the data described and information submitted to support registration of *Bacillus pumilus* strain GB34 require further clarification, justification or additional information for them to be considered complete and acceptable. The submission can be upgraded to acceptable with submission of adequate information/clarification for the deficiencies described below.

MRID 45433501 Acute Oral Toxicity Study in Rats--Limit Test: GB 34 Technical
CLASSIFICATION: UNACCEPTABLE
The oral LD_{50} of GB 34 Technical for male, female and male and female combined is >5000 mg/kg (toxicity category IV). No acute oral pathogenicity data were submitted to satisfy guideline 885.3050, Acute Oral Toxicity Pathogenicity. The test substance was indicated as being composed of *Bacillus subtilis*. Composition of test substance requires clarification. Additional data citations and/or scientifically supported waiver requests are needed to support guideline.

MRID 45433502 Primary Eye Irritation Study in Rabbits: GB 34 Technical
CLASSIFICATION: UNACCEPTABLE but upgradable
GB 34 Technical test substance was mildly irritating to the eye of New Zealand albino rabbits (toxicity category III). The maximum mean total scores was 9.3 at 1 hour post-dosing which cleared by 72 hours. The test substance is indicated as being composed of *Bacillus subtilis*. Identity of test substance requires clarification.

MRID 45433503 Primary Skin Irritation Study in Rabbits: GB 34 Technical
CLASSIFICATION: UNACCEPTABLE but upgradable
GB 34 Technical was non-irritating to the New Zealand rabbits (toxicity category IV). The test substance was indicated as being composed of *Bacillus subtilis*. Identity of test substance requires clarification.

RECOMMENDATIONS:

(1) A complete hazard assessment could not be performed because of insufficient toxicity and usage information for GB34 Technical Biological Fungicide. In MRIDs 454335-01, -02, and -03, it was indicated that the GB34 Technical test substance is composed of *Bacillus subtilis* whereas the active ingredient indicated in the registration application is *Bacillus pumilus* strain GB34. Further information identifying the genera, species an strain of the bacteria contained in the GB34 Technical test substance must be provided to address this discrepancy

(2) Additional data citations and/or scientifically supported waiver requests are needed to support the following guidelines:

885.3050, Acute Oral Toxicity Pathogenicity
885.3150 Acute Pulmonary Toxicity/Pathogenicity
885.3400 Hypersensitivity Incidents
870.1200 Acute Dermal Toxicity
870.1300 Acute Inhalation Toxicity
870.2600 Skin Sensitization
152-38 Immune Response.

The registrant is required to address each of the guideline requirements above. All waivers that are submitted need to include supporting scientific rationale. If scientific literature are cited, the registrant should provide the abstract and cited document. To facilitate the review of any supporting publications, it is preferable that the pertinent section of the document be clearly cross referenced to the appropriate guideline(s).

(3) The registrant did not submit an application to register an end-use product containing *Bacillus pumilus* Strain GB34. Information concerning the proposed use sites, application methods, and rates of application are requested to complete the risk assessment for this new active ingredient.

DATA EVALUATION REPORT

Reviewed by: Susanne Cerrelli SC 4/11/2002

Secondary Reviewer: Ibrahim S. Barsoum, Ph.D., Microbiologist ISB 4/11

STUDY TYPE:	Moore, G. (2001) Acute Oral Toxicity Study in Rats--Limit Test: GB 34 Technical: Lab Project Number: 10085: P320. Unpublished study prepared by Product Safety Labs. 14 p. {OPPTS 870.1100}
MRID NO:	454335-01
TEST MATERIAL:	GB 34 Technical (*Bacillus subtilis* 1.0 x 10^{10} cfu/g) Lot #P104:66-1
PROJECT NO:	10085: P320.
SPONSOR:	GUSTAFSON LLC
TESTING FACILITY:	Product Safety Labs 725 Cranbury Road East Brunswick, New Jersey 08816
TITLE OF REPORT:	Acute Oral Toxicity Study in Rats--Limit Test: GB 34 Technical
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	February 1, 2001
CONCLUSION:	The oral LD_{50} of GB 34 Technical for male, female and male and female combined is >5000 mg/kg. No data were provided to assess acute oral pathogenicity of test substance. The test substance was indicated as being composed of *Bacillus subtilis*. Composition of test substance requires clarification.
CLASSIFICATION:	UNACCEPTABLE TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: GB 34 Technical Lot #P104:66-1

Test Animals: Five male and five female Sprague-Dawley derived, albino rats were received from Ace Animals, Inc., Boyertown, PA and weighed 264-299 grams (male) and 200-220 grams (females) on the day of dosing. The rats were ear tagged with the numbers 9904-9908 (males) and 9909-9913 (females), housed individually in suspended stainless steel caging with mesh floors. The animal room maintained a temperature of 17-24°C. and a 12 hour light/dark cycle. The rats were supplied filtered water *ad libitum* and Purina Rat Chow #5012.

Methods: At the start of the study, each rat received a single 5,000 mg/kg gavage dose of GB 34 Technical, previously ground and diluted to 35% w/w with distilled water. Rats were weighed on days 0, 7 and 14. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at 1 and 3 hours post-dosing and at least once daily thereafter for 14 days. Observations include gross evaluation of skin, and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. At the end of the study all rats were euthanized by CO_2 inhalation, and necropsies were performed.

II. RESULTS

Mortality: No rats died during the study.

Body weights: All animals gained weight.

Clinical Observations: No clinical signs of toxicity were noted.

Gross Necropsy: No abnormal findings were noted at necropsy.

III. DISCUSSION

No morbidity, moribundity, or effects on body weight were found following treatment of rats with 5000 mg/kg test material. Therefore the Sprague Dawley rat oral LD_{50} of GB 34 Technical for male, female and male and female combined is >5000 mg/kg, placing the test material in TOXICITY CATEGORY IV. It appears that *Bacillus pumilus* strain GB34 was not tested. The report indicates that the test substance is composed of *Bacillus subtilus* 1.0×10^{10} cfu/g whereas the active ingredient indicated in the registration application is *Bacillus pumilus* strain GB34. Otherwise, the toxicity data are adequate. However, no data, or data waivers were provided to address acute oral pathogenicity data requirements outlined in Guideline OPPTS 885.3050. The packet classification is UNACCEPTABLE but UPGRADABLE. Additional information are required to address the test substance composition and to address acute oral pathogenicity guideline requirements.

DATA EVALUATION REPORT

Reviewed by: Susanne Cerrelli

Secondary Reviewer: Ibrahim S. Barsoum, Ph.D., Microbiologist ISB 4/19

STUDY TYPE:	Moore, G. (2001) Primary Eye Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10086: P324. Unpublished study prepared by Product Safety Labs. 16 p. {OPPTS 870.2400}
MRID NO:	454335-02
TEST MATERIAL:	GB 34 Technical (*Bacillus subtilis* 1.0 x 10^{10} cfu/g) Lot #P104:66-1
PROJECT NO:	10086: P324
SPONSOR:	GUSTAFSON LLC
TESTING FACILITY:	Product Safety Labs 2394 Route 130 Dayton, NJ 08810
TITLE OF REPORT:	Primary Eye Irritation Study in Rabbits: GB 34 Technical
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	February 14, 2001
GOOD LABORATORY PRACTICE:	GLP compliant except that characterization, stability, identity and verification of test substance concentration were not evaluated at Product Safety Labs.
CONCLUSION:	GB 34 Technical test substance was mildly irritating to the eye of New Zealand albino rabbits. The maximum mean total scores was 9.3 at 1 hour post-dosing which cleared by 72 hours. The test substance is indicated as being composed of *Bacillus subtilis* which requires clarification.
CLASSIFICATION:	Unacceptable- may be upgraded with additional information on test substance composition. TOXICITY CATEGORY III

I. STUDY DESIGN

Test Material: GB 34 Technical Lot #P104:66-1

Test Animals: Three male and three female young adult New Zealand albino rabbits were received Davidson's Mill Farm, South Brunswick, NJ Body weights were not provided. The rabbits were ear tagged with the numbers 4080-4085 (males even numbered), housed individually in suspended stainless steel caging with mesh floors. The rabbits were quarantined 6 days prior to treatment and the animal room maintained a temperature of 19-22°C, and a 12 hour light/dark cycle. The rabbits were supplied filtered water *ad libitum* and Purina Rabbit Chow #5326.

Methods: The test material was ground prior to instillation. The ground test material 0.1 mL (0.07-0.08 g) was instilled int o the everted lower lid of the right eye and the upper and lower lids were gently closed together for one second. The contralateral eye served as a control The eyes were examined and scored according to the Draize method at 1, 24, 48 and 72 hours after instillation. The 24 hour examination also included a fluorescein dye evaluation for corneal effects.

II. RESULTS

Mortality: All rabbits survived the study

Ocular Lesions: No corneal opacity or iritis were observed. Within one hour of treatment all rabbits developed conjunctivitis (score= ranging from 8 to 10) that resolved within 72 hours. The maximum ocular score was 10 recorded at one hour after test material instillation based on conjunctivae observations. The maximum mean total score was 9.3.

Scale for Scoring Ocular Lesions (Draize technique)

Cornea

A. Opacity-degree of density (area most dense taken for reading)
- No opacity
- Scattered or diffuse area of opacity, details of iris clearly visible
- Easily discernible translucent areas, details of iris slightly obscured
- Opalescent areas, no details of iris visible, size of pupil barely discernible
- Opaque, iris invisible

B. Area of cornea involved
- One quarter (or less) but not zero
- Greater than one quarter, but less than half
- Greater than half, but less than three quarters
- Greater than three quarters, up to whole area

Score=A x B x 5 Total Maximum Score=80

Iris

A. Values

Normal	0
Folds above normal, congestion, swelling circumcorneal injection (any and all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these).	2

Score=Ax 5 Total Maximum Score=10

Conjunctive

A. Redness (refers to palpebral and bulbar conjunctive excluding cornea and iris)

Blood vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

B. Chemosis:

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

C. Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score =(A+B+ C) x 2 Total Maximum=20

III. DISCUSSION

Based on the presented data, all rabbits developed moderate conjunctival irritation that cleared up within 72 hours of treatment. No corneal opacity of iritis or non-ocular effects were noted. The GB 34 Technical test substance was mildly irritating to the eye and is placed in **Toxicity Category III.** The packet classification is **unacceptable but upgradable** because it appears that *Bacillus pumilus* strain GB34 was not tested. The report indicates that the test substance is composed of *Bacillus subtilis* 1.0 x 10^{10} cfu/g whereas the active ingredient indicated in the registration application is *Bacillus pumilus* strain GB34. Further information must be provided to address this discrepancy.

DATA EVALUATION REPORT

Reviewed by: Susanne Cerrelli SC 4/11/2002

Secondary Reviewer: Ibrahim S. Barsoum, Ph.D., Microbiologist ISB 4/11/02

STUDY TYPE:	Citation: Moore, G. (2001) Primary Skin Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10087: P326. Unpublished study prepared by Product Safety Labs. 15 p. {OPPTS 870.2500}
MRID NO:	45433503
TEST MATERIAL:	GB 34 Technical (*Bacillus subtilis* 1.0 x 10^{10} cfu/g) Lot #P104:66-1
PROJECT NO:	10087: P326.
SPONSOR:	GUSTAFSON LLC
TESTING FACILITY:	Product Safety Labs 2394 Route 130 Dayton, NJ 08810
TITLE OF REPORT:	Primary Skin Irritation Study in Rabbits: GB 34 Technical
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	February 1, 2001
GOOD LABORATORY PRACTICE:	GLP compliant except that characterization, stability, identity and verification of test substance concentration were not evaluated at Product Safety Labs.
CONCLUSION:	GB 34 Technical was non-irritating to the New Zealand rabbits. The test substance was indicated as being composed of *Bacillus subtilis* which requires clarification.
CLASSIFICATION:	Unacceptable- may be upgraded with additional information on test substance composition. TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: GB 34 Technical Lot #P104:66-1

Test Animals: Three male and three female young adult New Zealand albino rabbits were received Davidson's Mill Farm, South Brunswick, NJ. Body weights were not provided. The rabbits were ear tagged with the numbers 4028-4033 (males even numbered), housed individually in suspended stainless steel caging with mesh floors. The rabbits were quarantined 6 days prior to treatment and the animal room maintained a temperature of 19-21°C, and a 12 hour light/dark cycle. The rabbits were supplied filtered tap water *ad libitum* and Purina Rabbit Chow #5326

Methods: On the day before application the dorsal area and the trunk of each of the animals was clipped. The rabbits were given a single 0.5 g dose of test material (equivalent to a 0.71 g dry paste when moistened with distilled water to achieve a 70% w/w mixture) under a one inch 1 inch x 1 inch gauze pad on a 6 cm^2 clipped site. The gauze pad was secured with 3" Micropore tape wrapped around the trunk. Elizabethan collars were placed on the animals. 4 hours later the collars and coverings were removed and the sites were wiped with water and a clean towel to remove any residual test substance. The application sites were observed at 1, 24, 48, and 72 hours after patch removal. In addition the animals were observed for signs of behavioral changes and gross toxicity at least once during the 72 hour test period.

II. RESULTS

Mortality: All rabbits survived the study

Clinical observations and dermal responses: No clinical signs of toxicity or dermal irritation were observed during the 72 hour study period.

Irritation Scores: no erythema or edema was observed on the treated sites during the 72 hour study

III. DISCUSSION

No dermal irritation was observed in the rabbits at any site. Based on the study results, GB 34 Technical is placed in **Toxicity Category IV.** The packet classification is **unacceptable but upgradable** because it appears that *Bacillus pumilus* strain GB34 was not tested.. The report indicates that the test substance is composed of *Bacillus subtilus* 1.0×10^{10} cfu/g whereas the active ingredient indicated in the registration application is *Bacillus pumilus* strain GB34. Further information must be provided to address this discrepancy.

DATA EVALUATION REPORT

Reviewed by: Susanne Cerrelli

Secondary Reviewer: Ibrahim S. Barsoum, Ph.D., Microbiologist ISB 4/9

STUDY TYPE:	Moore, G. (2001) Primary Eye Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10086: P324. Unpublished study prepared by Product Safety Labs. 16 p. {OPPTS 870.2400}
MRID NO:	454335-02
TEST MATERIAL:	GB 34 Technical (*Bacillus subtilis* 1.0 x 10^{10} cfu/g) Lot #P104:66-1
PROJECT NO:	10086: P324
SPONSOR:	GUSTAFSON LLC
TESTING FACILITY:	Product Safety Labs 2394 Route 130 Dayton, NJ 08810
TITLE OF REPORT:	Primary Eye Irritation Study in Rabbits: GB 34 Technical
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	February 14, 2001
GOOD LABORATORY PRACTICE:	GLP compliant except that characterization, stability, identity and verification of test substance concentration were not evaluated at Product Safety Labs.
CONCLUSION:	GB 34 Technical test substance was mildly irritating to the eye of New Zealand albino rabbits. The maximum mean total scores was 9.3 at 1 hour post-dosing which cleared by 72 hours. The test substance is indicated as being composed of *Bacillus subtilis* which requires clarification.
CLASSIFICATION:	Unacceptable- may be upgraded with additional information on test substance composition. TOXICITY CATEGORY III

I. STUDY DESIGN

Test Material: GB 34 Technical Lot #P104:66-1

Test Animals: Three male and three female young adult New Zealand albino rabbits were received Davidson's Mill Farm, South Brunswick, NJ . Body weights were not provided. The rabbits were ear tagged with the numbers 4080-4085 (males even numbered), housed individually in suspended stainless steel caging with mesh floors. The rabbits were quarantined 6 days prior to treatment and the animal room maintained a temperature of 19-22°C, and a 12 hour light/dark cycle. The rabbits were supplied filtered water *ad libitum* and Purina Rabbit Chow #5326.

Methods: The test material was ground prior to instillation. The ground test material 0.1 mL (0.07-0.08 g) was instilled int o the everted lower lid of the right eye and the upper and lower lids were gently closed together for one second. The contralateral eye served as a control The eyes were examined and scored according to the Draize method at 1, 24, 48 and 72 hours after instillation. The 24 hour examination also included a fluorescein dye evaluation for corneal effects.

II. RESULTS

Mortality: All rabbits survived the study.

Ocular Lesions: No corneal opacity or iritis were observed. Within one hour of treatment all rabbits developed conjunctivitis (score= ranging from 8 to 10) that resolved within 72 hours. The maximum ocular score was 10 recorded at one hour after test material instillation based on conjunctivae observations. The maximum mean total score was 9.3.

Scale for Scoring Ocular Lesions (Draize technique)

Cornea

A.	Opacity-degree of density (area most dense taken for reading)	
	No opacity	0
	Scattered or diffuse area of opacity, details of iris clearly visible	1
	Easily discernible translucent areas, details of iris slightly obscured	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible	3
	Opaque, iris invisible	4
B.	Area of cornea involved	
	One quarter (or less) but not zero	1
	Greater than one quarter, but less than half	2
	Greater than half, but less than three quarters	3
	Greater than three quarters, up to whole area	4

Score=A x B x 5 Total Maximum Score=80

Iris

A.	Values	
	Normal	0
	Folds above normal, congestion, swelling circumcorneal injection (any and all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
	No reaction to light, hemorrhage, gross destruction (any or all of these).	2
	Score=Ax 5 Total Maximum Score=10	

Conjunctive

A.	Redness (refers to palpebral and bulbar conjunctive excluding cornea and iris)	
	Blood vessels normal	0
	Vessels definitely injected above normal	1
	More diffuse, deeper crimson red, individual vessels not easily discernible	2
	Diffuse beefy red	3
B.	Chemosis	
	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	2
	Swelling with lids about half closed	3
	Swelling with lids about half closed to completely closed	4
C.	Discharge	
	No discharge	0
	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
	Discharge with moistening of the lids and hairs just adjacent to lids	2
	Discharge with moistening of the lids and hairs, and considerable area around the eye	3
	Score =(A+B+ C) x 2 Total Maximum=20	

III. DISCUSSION

Based on the presented data, all rabbits developed moderate conjunctival irritation that cleared up within 72 hours of treatment. No corneal opacity of iritis or non-ocular effects were noted. The GB 34 Technical test substance was mildly irritating to the eye and is placed in **Toxicity Category III.** The packet classification is **unacceptable but upgradable** because it appears that *Bacillus pumilus* strain GB34 was not tested. The report indicates that the test substance is composed of *Bacillus subtilus* 1.0 x 10^{10} cfu/g whereas the active ingredient indicated in the registration application is *Bacillus pumilus* strain GB34. Further information must be provided to address this discrepancy.

13544

R144582

Chemical: Bacillus pumilus GB34

PC Code:
006493

HED File Code: 41500 BPPD Tox/Chem
Memo Date: 4/11/2002
File ID: DPD279234
Accession #: 000-00-9002

HED Records Reference Center
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