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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

Memorandum

OCT 15 2002

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 as well as Revisions to Manufacturing Process Submitted by Encore Technologies, 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: Carl Etsitty, M.S., Microbiologist
Microbial Pesticide Branch,
Biopesticides and Pollution Prevention Division (7511C)

Thru: John L. Kough, Ph.D., Senior Scientist
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, primary skin irritation study in rabbits; and review waiver of several of the toxicity data requirements justification for several toxicity data requirements, submitted by Gustafson LLC, and secondly, review revisions to the manufacturing process submitted by Encore Technologies, 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 and 7501-ROR
Chemical Number: 006493
Submission Number: S606318 and S620265
DP Barcode: D279234 and D285138
MRID No:

45722501 Moore, G. (2002) Acute Oral Toxicity Study in Rats – Limit Test: GB 34 Technical: Lab Project Number: 10085. Unpublished study prepared by Product Safety Labs. 15 p. {OPPTS 870.1100}

45722502 Moore, G. (2002) Primary Eye Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10086. Unpublished study prepared by Product Safety Labs. 17 p. {OPPTS 870.2400}

45722503 Moore, G. (2002) Primary Skin Irritation Study in Rabbits: GB 34 Technical: Lab Project Number: 10087. Unpublished study prepared by Product Safety Labs. 16 p. {OPPTS 870.2500}

45723401 Richards, S J. (2002) Revisions to GB34 TGAI Manufacturing Process; Lab Project Number: None given. Unpublished study prepared by Encore Technologies, LLC. 4 p {OPPTS 885.1200}

No MRID No Author (2002). Acute Oral Toxicity/Pathogenicity – Wavier Request. Lab Project Number: None given. Unpublished study prepared by Encore Technologies, LLC. {OPPTS 885.3150}

No Author (2002). Acute Dermal Toxicity/Pathogenicity – Wavier Request. Lab Project Number: None given. Unpublished study prepared by Encore Technologies, LLC. {OPPTS 885.3100}

No Author (2002). Acute Pulmonary Toxicity/Pathogenicity – Wavier Request. Lab Project Number: None given. Unpublished study prepared by Encore Technologies, LLC. {OPPTS 885.3050}

BACKGROUND:

B. pumilus is a naturally occurring soil microorganism that acts 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).

Gustafson LLC has a currently registered strain of *B. subtilis* which has a complete toxicity package. The *Bacillus pumilus* organism was claimed by Gustafson to be very similar to their registered strain of *B. subtilis*. In addition, the company provided a set of acute toxicity data for the technical and concentrate as well as a injection toxicity/infectivity test to be able to bridge GB34 to their *B. subtilis* data base. In order to complete the review for the tolerance determination, the applicability of the data waivers for the other toxicity/infectivity tests needs to be determined.

Furthermore, 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.

The submitted data is supersedes MRID# 45433501-03. The data are similar with one clarification as stated in MRID# 45722500 "The reports state on one page that eh material tested is bacillus subtilis (sic), while it was actually bacillus pumilus (sic). The amended reports correct the topographical error." It further addresses the supplemental conditions including the pathogenicity data wavier request as well as 2 of 4 batch lot analysis.

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 45722501 Acute Oral Toxicity Study in Rats – Limit Test: GB 34 Technical
CLASSIFICATION: ACCEPTABLE, Toxicity Category IV
CONCLUSION: The oral LD_{50} of GB 34 Technical for male, female and male and female combined is >5000 mg/kg.

MRID 45722502 Primary Eye Irritation Study in Rabbits: GB 34 Technical
CLASSIFICATION: ACCEPTABLE, Toxicity Category III

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

MRID 45722503 Primary Skin Irritation Study in Rabbits: GB 34 Technical
CLASSIFICATION: ACCEPTABLE, Toxicity Category IV
CONCLUSION: GB 34 Technical was non-irritating to the New Zealand rabbits.

MRID 45723401 Revisions to GB34 TGAI Manufacturing Process
CLASSIFICATION: SUPPLEMENTAL – Up gradable with clarification of spore counts vs. CFUs, and submission of methods.
CONCLUSION: Two of the 4 batches requested has been submitted. The data shows a consistence 10^{11} CFU counts, with identified contaminant levels below the registrant's manufacturing set contaminant levels. [redacted]

The literature provided as well as the results of the toxicity tests submitted to date do not indicate that the *B. pumilus* strain GB34 is toxic or infective. Moreover the results would suggest that the GB34 strain does not express the 6500 molecular weight toxin discussed in two papers nor does it exhibit any infectivity potential by the intravenous route as found in the clinical reports.

MRID None Wavier Request – Acute Oral/Dermal/Pulmonary Toxicity and Pathogenicity
CLASSIFICATION: SUPPLEMENTAL
CONCLUSION: Waiver requests for the dermal, oral and pulmonary toxicity/infectivity tests would support a finding of a reasonable certainty of causing no harm for a seed use of *B. pumilus* GB34. For other uses, especially with greater pulmonary exposure, the company must more definitively establish why the *B. subtilis* GB03 and *B. pumilus* GB34 are taxonomically so closely related to justify using the pulmonary infectivity test from GB03 to assess this endpoint for GB34.

RECOMMENDATION: The link between the company's *B. subtilis* GB03 and the GB34 strain of *B. pumilus* has not been clearly established. An explanation of spore counts vs. CFUs, and submission of methods. Lastly, provide a MRID number for waiver request data submission

*** Claimed confidential by submitter***

Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John Kough, Ph.D., Senior Scientist

STUDY TYPE:	Acute Oral Toxicity - Rats (OPPTS 870.1100)
MRID NO:	457225-01
TEST MATERIAL:	GB 34 Concentrate (~2% *Bacillus pumilus*)
PROJECT NO:	10085
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY:	Product Safety Labs, East Brunswick, NJ
TITLE OF REPORT:	Acute Oral Toxicity Study in Rats – Limit Test
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	July 19, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant except for characterization and stability of the test substance
CONCLUSION:	The oral LD_{50} of GB 34 Concentrate for male, female, and male and female rats is >5000 mg/kg.
CLASSIFICATION:	ACCEPTABLE – TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: GB 34 Concentrate (~2% *Bacillus pumilus*, 98% inert ingredients) Lot # P104-66-1 containing ~1×10^{13} cfu/g, with PSL reference number E01219-7R.

Test Animals: Five male and five female young adult Sprague-Dawley rats were received from Ace Animals, Inc., Boyertown, PA, and weighed 181-202 g (males) and 160-165 g (females) on the day of dosing. The rats were ear-tagged with the numbers 6088-6092 (males) and 6093-6097 (females), housed individually in stainless steel cages with wire mesh floors, and quarantined for 8 days before the start of the study. The animal room was controlled at 18-24°C with a 12 hour light/dark cycle. The rats received Purina Rodent Chow #5012 and filtered tap water *ad libitum*.

Methods: At the start of the study, each rat received a single 5000 mg/kg gavage dose of the GB 34 Concentrate, previously diluted to a 40% w/w solution with distilled water, at a dosing volume of 1 mL/100 g. The rats were observed for morbidity, moribundity, and behavioral changes 1 and 3 hours after dosing and at least daily thereafter for 14 days. They were weighed on days 0, 7, and 14. At the end of the study, the rats were euthanized by CO_2 inhalation and necropsied.

II. RESULTS

Mortality: No rats died during the study.

Body Weights: All animals gained weight.

Clinical Observations: No clinical signs of toxicity were observed.

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 Concentrate for male, female, and male and female combined is >5000 mg/kg, placing the test material in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE**.

DATA EVALUATION REPORT

Reviewed by: Carl Etsity, M.S., Microbiologist

Secondary Reviewer: John Kough, Ph.D., Senior Scientist

STUDY TYPE:	Primary Dermal Irritation - Rabbits (OPPTS 870.2500)
MRID NO:	452940-03
TEST MATERIAL:	GB 34 Concentrate (~2% *Bacillus pumilus*)
PROJECT NO:	10087
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY	Product Safety Labs, East Brunswick, NJ
TITLE OF REPORT:	Primary Skin Irritation Study in Rabbits
AUTHOR(S):	George E. Moore, B.S
STUDY COMPLETED:	July 19, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant except for characterization and stability of the test substance
CONCLUSION:	GB 34 Concentrate was nonirritating to the New Zealand white rabbit
CLASSIFICATION:	ACCEPTABLE – TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: GB 34 Concentrate (~2% *Bacillus pumilus*, 98% inert ingredients) Lot #P104-66-1 containing ~1 × 10^{10} cfu/g.

Test Animals: Three male and three female young adult New Zealand white rabbits were received from Davidson's Mill Farm, South Brunswick, NJ. Body weights not reported. The rabbits were ear-tagged with the numbers 1963-1968 (males odd number) and housed individually in stainless steel cages with mesh floors. They received filtered tap water and diet (Pelleted Purina Rabbit Chow #5326) *ad libitum*. The animals were quarantined for 11 days prior to treatment and the animal room was controlled at 19-27°C with a 12 hour light/dark cycle.

Methods: At the time of the study, the fur on the dorso-lumbar area of each rabbit was clipped. The rabbits were given a single 0.5 g dose of test material (equivalent to 0.77 g when moistened to a 65% w/w mixture with distilled water) applied under a 1 inch × 1 inch 4-ply 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. Four hours later, the collar and covering were removed and the site wiped with a moistened towel. The application sites were observed for dermal irritation 1, 24, 48, and 72 hours after patch removal. In addition, the rabbits were observed at least daily for clinical signs of toxicity during the 72-hour study period.

II. RESULTS

Mortality: All rabbits survived the study.

Clinical Observation 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.

Description of rating method.

Evaluation of Skin Reaction	Score
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing erythema reading	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised by more than 1.0 mm extending beyond the area of exposure)	4

III. DISCUSSION

No dermal irritation was observed on any rabbit at any test site. Based on the study results, GB 34 is nonirritating to the New Zealand white rabbit and is placed in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION REPORT	
Reviewed by	Carl Etsitty, M.S., Microbiologist
Secondary Reviewer:	John Kough, Ph.D., Senior Scientist
STUDY TYPE:	Primary Eye Irritation - Rabbits (OPPTS 870.2400)
MRID NO:	457225-02
TEST MATERIAL:	GB 34 Concentrate (~2% *Bacillus pumilus*)
PROJECT NO:	10086
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY:	Product Safety Labs, East Brunswick, NJ
TITLE OF REPORT:	Primary Eye Irritation Study in Rabbits
AUTHOR(S):	George E. Moore, B.S.
STUDY COMPLETED:	July 19, 2002
GOOD LABORATORY PRACTICE:	GLP Compliant except for characterization and stability of test substance
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.
CLASSIFICATION:	ACCEPTABLE – TOXICITY CATEGORY III

I STUDY DESIGN

Test Material: GB 34 Concentrate (~2% *Bacillus pumilus*, 98% inert ingredients) Lot # P104:66-1 containing ~1 × 10^{10} cfu/g.

Test Animals: Three male and three female young adult New Zealand white rabbits were received from Davidson's Mill Farm, South Brunswick, NJ. Body weights were not provided. The rabbits were ear-tagged with numbers 2021-2026 (males odd numbers) and housed individually in metal cages. They received filtered tap water and diet (Pelleted Purina Rabbit Chow #5326) *ad libitum*. They were quarantined 5 days prior to treatment and the animal room was controlled at 18-24°C with a 12 hour light/dark cycle. Prior to test material instillation , both eyes were treated with 2% fluorescein and examined under UV light for ocular abnormalities.

Methods: The test material, 0.1 mL (equivalent to 0.05-0.07 g), was instilled into the everted lower lid of the right eye and the upper and lower lids held closed for 1 second. The contralateral eye served as control. The eyes were examined and scored according to the Draize method 1, 24, 48 and 72 hours after test material instillation. The 24 hour examination also included a fluorscein staining examination 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 mild conjunctival irritation (score = 1) that resolved on all animals within 48 hours of treatment. The maximum ocular irritation score was 4.7 recorded one hour after test material instillation.

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, 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 tree quarters . . . 3
   - Greater than three quarters, up to whole area . . . 4

   Score = A × B × 5. Total Maximum Score = 80

Iris

A. Values
   - Normal . . . 0
   - Folds above normal, congestion, swelling, circumcorneal injection (any or 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 = A × 5. Total Maximum Score = 10

Conjunctiva

A. Redness (refers to palpebral and bulbar conjunctive excluding cornea and iris)
   - 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 x B × C) × 2. Total Maximum Score = 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 **ACCEPTABLE.**

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	457234-01
TEST MATERIAL:	GB 34 TGAI (*Bacillus pumilus*)
PROJECT NO:	None Given
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY:	Encore Technologies[1], LLC, Plymouth, MN
TITLE OF REPORT:	Revisions to GB34 TGAI Manufacturing Process
AUTHOR(S):	Sharon J. Richards
STUDY COMPLETED:	June 25, 2002
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	Two of the 4 batches requested has been submitted. The data shows a consistence 10^{11} CFU counts, with identified contaminant levels below the registrant's manufacturing set contaminant levels [redacted]
CLASSIFICATION:	Supplemental – Upgradable with clarification of spore counts vs. CFUs, and submission of methods.

– CONTAINS CONFIDENTIAL BUSINESS INFORMATION –

1. STUDY DESIGN

*** Claimed confidential by submitter***

Manufacturing process information may be entitled to confidential treatment

Test Material: GB 34 Concentrate

Discusion of Enforcement Method for Batches that do not meet Specifications

[redacted]

Viable spore numbers: Ensure that the final TGAI viable spore number per gram is greater than 1.0×10^{11} CFU

[1]Encore Technologies, LLC has requested the manufacturing process kept confidential from the applicant, Gustafson LLC. June 19, 2002, Encore Technologies to Environmental Protection Agency, Phil Hutton.

Microbial identity: GB34 TGAI is submitted for FAME analysis by an outside lab.

METHODS

Non given.

RESULTS

Test Material: GB 34 Concentrate, Lot No. 00GUS13-06 and 000920

[redacted]

III. DISCUSSION

The submitted data is in response to Etsitty to Ball, February 4, 2002, memorandum. The registrant has attempted to address MRID 454603-01 "Supplemental: Up gradable to Acceptable with the following justification/clarification – Additional 4 batch analysis, including a discussion of the enforcement method for batches that do not meet QA/QC for AI amounts or microbial contaminants."

Two of the 4 batches requested has been submitted. The data shows a consistence 10^{11} CFU counts, with identified contaminant levels below the registrant's manufacturing set contaminant levels. [redacted] The packet is Supplemental with a clarification of viable spore numbers vs. CFU needs a clear distinctions; also, submission of methods.

*** Claimed confidential by submitter***
Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John Kough, Ph.D., Senior Scientist

STUDY TYPE:	Wavier Request
MRID NO:	None Given
TEST MATERIAL:	GB 34 Concentrate (~2% *Bacillus pumilus*)
PROJECT NO:	None Given
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY:	Gustafson LLC, Plano, TX
TITLE OF REPORT:	Acute Oral Toxicity/Pathogenicity Acute Pulmonary Toxicity/Pathogenicity Acute Dermal Toxicity/Pathogenicity
AUTHOR(S):	None Given
STUDY COMPLETED:	None Given
GOOD LABORATORY PRACTICE:	Non GLP
CONCLUSION:	Waiver requests for the dermal, oral and pulmonary toxicity/infectivity tests would support a finding of a reasonable certainty of causing no harm for a seed use of *B. pumilus* GB34. For other uses, especially with greater pulmonary exposure, the company must more definitively establish why the *B. subtilis* GB03 and *B. pumilus* GB34 are taxonomically so closely related to justify using the pulmonary infectivity test from GB03 to assess this endpoint for GB34
CLASSIFICATION:	Supplemental

I. STUDY DESIGN

Test Material: GB 34 Concentrate (~2% *Bacillus pumilus*, 98% inert ingredients)

BACKGROUND: Gustafson LLC has a currently registered strain of *B. subtilis* which has a complete toxicity package. The *Bacillus pumilus* organism was claimed by Gustafson to be very similar to their registered strain of *B. subtilis*. In addition, the company provided a set of acute toxicity data fro the technical and concentrate as well as a injection toxicity/infectivity test to be able to bridge GB34 to their *B. subtilis* data base. In order to complete the review for the tolerance determination, the applicability of the data waivers for the other toxicity/infectivity tests needs to be determined.

DISCUSSION: The literature provided as well as the results of the toxicity tests submitted to date do not indicate that the *B. pumilus* strain GB34 is toxic or infective. Moreover the results would suggest that the GB34 strain does not express the 6500 molecular weight toxin discussed in two papers nor does it exhibit any infectivity potential by the intravenous route as found in the clinical reports

RECOMMENDATION: The link between the company's *B. subtilis* GB03 and the GB34 strain of *B. pumilus* has not been clearly established.

SUMMARY OF DATA SUBMITTED: The published literature provided as results from a literature search for adverse human health effects from exposure to *Bacillus pumilis* did not indicate that the GB34 strain under consideration had any connection to the reported incidents. The literature ranged from clinical reports to research on toxin production from isolated strains. A short summary of each paper follows.

F.E. Berkowitz (1994) The gram-positive bacilli: a review of the microbiology, clinical aspects and antimicrobial susceptibilities of a heterogenous group of bacteria, Pediatric Infectious Disease Journal 13:1126-38. This articles states that the major cause of non-anthrax bacillus infections are due to *B. cereus*. Other reported *Bacillus* species are almost invariably associated with bacteremia after the host defenses have been weakened or breached. This includes cases involving trauma, surgery, in-dwelling catheters, intravenous drug use, cancer and tracheal intubation and is confirmed in the other reports submitted. The only direct mention of *B. pumilus* is mention of it being responsible for food poisoning.

S.K. Pool & A.J. Smally, Seizure in a Five-year-old, Hospital Practice, March 15, 1993, p.110-104. This presents a clinical case presentation of seizures in a young boy in an incident of *B. pumilus* sepsis and possible food poisoning. While the original suspected organism was *B. cereus*, later confirmation states *B. pumilus* and probable *B. licheniformis*. This indicates a inconclusive identification by the microbiology lab but the main point is that the authors claim that the report indicates that *B. cereus/B. pumilus* can cause true sepsis syndrome. Given the context of the child's treatment for otitis media with amoxicillin and dexamethasone, he could have been immune compromised which exacerbated the infection.

K.A. Workowski & J.P. Flaherty (1992) Systemic Bacillus Species Infection Mimicing Listeriosis of Pregnancy Clinical Infectious Diseases 14:694-696. A clinical case report of a 23-year old pregnant intravenous cocaine user whose *Bacillus* bacteremia lead to premature labor. The patient was successfully treated with gentamicin and ampicillin and a child was delivered by Cesarean section. The placenta exhibited severe acute villitis with numerous gram positive bacilli present. Vitek identification indicated the bacterium to be *Bacillus pumilus*.

D.I. Bernstein, Z.L. Lummus, G. Santilli, J. Sisosky & I.L. Bernstein (1995) Machine Operator's Lung, A Hypersensitivity Pneumonitis Disorder Associated With Exposure to Metalworking Fluids Aerosols, Chest 108:636-41. This is a case report of six auto parts manufacturers who developed hypersensitive pneumonitis from bacteria present and apparently growing in an aqueous metalworking fluid. The bacteria isolated from the fluid and shown to have precipitins with the workers sera included *Pseudomonas fluorescens, Aspergillus niger, Staphylococcus capitas, Rhodococcus* and *Bacillus pumilus*

B. Hoult & A.F. Tuxford (1991) Toxin Production by *Bacillus pumilus*, J. of Clinical Pathology 44:455-458. Two strains of *B. pumilus* (M11 and M38) were isolated from a Lancashire cotton mill's air and shown to have cytopathic effects on Vero cell cultures (green monkey kidney cells). The cell free supernatant of one strain was shown to have cytotoxic effects leading to mortality of the Vero cells after 96 hour incubation whereas the other gave cytopathic effects but

all the Vero cells survived. Both strains were shown to have lecithin and casein hydrolysis activity as well as the toxin but a single protein entity was not identified as the active moiety.

P.F. Brophy &F.C. Knoop (1982) *Bacillus pumilus* in the Induction of Clindamycin-Associated enterocolitis in Guinea Pigs, Infection and Immunity 35:289-295. This report describes efforts to isolate the causative agent responsible for enterocolitis that is associated with clindamycin treatment. A *B. pumilus* strain was isolated from the intestinal tract of guinea pigs with clindamycin induced enterocolitis. The strain was found to produce a toxin that mimicked but was not identical to the toxin produced by *Clostridium difficile*. The cell free filtrate was shown to have proteolytic and toxic activity that could be separated. The toxin weighed about 6500 daltons and was unaffected by DNase or RNase but was inactivated by lipase, trypsin or pronase. These results suggest that the toxin could be a lipoprotein.

In addition to the papers furnished above, the company provided rationales for waiving the oral, dermal and pulmonary toxicity/infectivity studies based on the lack of exposure due to the seed treatment use, protective clothing for applicators and the low residues found in the residue study. The company also cites the lack of adverse effects seen in their submitted studies of *B. pumilus* GB34 for intravenous injection toxicity/infectivity, for acute oral toxicity, skin and eye irritation using the technical and concentrate.

These justifications as well as the lack of toxicity/infectivity in the intravenous injection assay would justify waiving the pathogenicity tests for a seed treatment product. It is not clear from the submission how the *B. pumilus* GB34 strain was tied to the *B. subtilis* GB03 strain as the referenced MRID 452940-04 did not establish the similarity of these two bacteria.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L Kough, Ph.D., Senior Scientist

STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	457234-01
TEST MATERIAL:	GB 34 TGAI (*Bacillus pumilus*)
PROJECT NO:	None Given
SPONSOR:	Gustafson LLC, Plano, TX
TESTING FACILITY:	Encore Technologies[1], LLC, Plymouth, MN
TITLE OF REPORT:	Revisions to GB34 TGAI Manufacturing Process
AUTHOR(S):	Sharon J. Richards
STUDY COMPLETED:	June 25, 2002
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	Two of the 4 batches requested has been submitted. The data shows a consistence 10^{11} CFU counts, with identified contaminant levels below the registrant's manufacturing set contaminant levels. [redacted]
CLASSIFICATION:	Supplemental – Up gradable with clarification of spore counts vs. CFUs, and submission of methods.

– CONTAINS CONFIDENTIAL BUSINESS INFORMATION –

*** Claimed confidential by submitter***

Manufacturing process information may be entitled to confidential treatment

I. STUDY DESIGN

Test Material: GB 34 Concentrate

Discusion of Enforcement Method for Batches that do not meet Specifications

[redacted]

Viable spore numbers: Ensure that the final TGAI viable spore number per gram is greater than 1.0×10^{11} CFU.

[1]Encore Technologies, LLC has requested the manufacturing process kept confidential from the applicant, Gustafson LLC, June 19, 2002, Encore Technologies to Environmental Protection Agency. Phil Hutton.

Microbial identity: GB34 TGAI is submitted for FAME analysis by an outside lab.

METHODS

Non given.

RESULTS

Test Material: GB 34 Concentrate, Lot No. 00GUS13-06 and 000920

[redacted]

III. DISCUSSION

The submitted data is in response to Etsitty to Ball, February 4, 2002, memorandum. The registrant has attempted to address MRID 454603-01 "Supplemental: Up gradable to Acceptable with the following justification/clarification – Additional 4 batch analysis, including a discussion of the enforcement method for batches that do not meet QA/QC for AI amounts or microbial contaminants."

Two of the 4 batches requested has been submitted. The data shows a consistence 10^{11} CFU counts, with identified contaminant levels below the registrant's manufacturing set contaminant levels. [redacted] The packet is Supplemental with a clarification of viable spore numbers vs. CFU needs a clear distinctions; also, submission of methods.

*** Claimed confidential by submitter***

Manufacturing process information may be entitled to confidential treatment

13544

R147587

Chemical: Bacillus pumilus GB34

PC Code:
006493

HED File Code: 41500 BPPD Tox/Chem
Memo Date: 10/15/2002
File ID: DPD279234
Accession #: 000-00-9002

HED Records Reference Center
6/28/2007