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

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ecological risk Assessment for *Bacillus pumilus* GB34 Concentrate Biological Fungicide, EPA file symbol 007501-ROR from Gustafson LLC for Soybean Seed Treatment.

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DP Barcode: D285138
Case No.: 070592
MRID#'s: 452940-05
OPP Chemical Code: 006493
Pesticide Name: *Bacillus pumilus* GB34 Concentrate Biological Fungicide
Registrant: Gustafson LLC, 1400 Preston Road, Suite 400, Plan0, Texas 75093

I. PROPOSED USES:

To be used as seed treatment for the suppression of root diseases of soybeans caused by *Rhizoctonia* and *Fusarium*. BG34 Concentrate contains bacteria which colonize the developing root system, suppressing disease organisms that attack root systems. As the root system develops, the bacteria grow with the roots extending the protection throughout the growing season.

Methods of application and use rates

Bacillus pumilus Gb 34 Concentrate is applied as a water-based slurry either alone or with other registered seed treatment insecticides and fungicides through standard slurry or mist commercial seed treatment equipment. The application rate is 0.1 ounces per 100 pounds of seed amounting to approximately 2.84 x 10^{10} GB34 endospores/100 pounds of seed. At soybean planting rate of

70 pounds per acre the BG34 endospore CFU will be 2 x10^{10} per acre. The natural occurrence of bacilli in an acre of soil is estimated to be greater than 9.25 x 10^{17}. There fore the addition from the proposed soybean seed treatment uses is insignificant.

II. ENVIRONMENTAL EFFECTS

Data Waiver Requests: Ecological Effects

The registrant submitted written data waiver justifications for the following Tier I OPPTS guideline requirements based on the seed treatment use of *Bacillus pumilus* GB34 Concentrate:

Guideline	Study	Status
885.4100	Avian pulmonary/inhalation toxicity/pathogenicity	Not required for microbial pesticides not related to avian pathogens
885.4200	Freshwater fish toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.
885.4240	Freshwater Aquatic Invertebrate toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.
885.4150	Wild Mammal toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.
885.4300	Nontarget Plant toxicity/pathogenicity	Not required for microbial pesticides not related to plant pathogens
885.4340	Nontarget Insect toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.
885.4380	Honey Bee toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.
885.4280	Estuarine and Marine Animals toxicity/pathogenicity	Data requirement waived for seed treatment uses. See attached waiver justification.

A. Non-target species effects

The Agency has performed an environmental risk assessment and determined that the soybean seed treatment uses of GB34 biofungicide will have no adverse effects to avian species, including waterfowl which is most likely to be exposed: freshwater and estuarine/marine fish and invertebrates, terrestrial and aquatic insects, including the honey bee, and terrestrial and aquatic

plant species. Because BG34 biofungicide does not pose a hazard to wildlife at the proposed use rates, there also is no "may effect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

Bacillus pumilus belongs to the *subtilis* group of bacilli and is an ubiquitous bacterium commonly found in soil, water, air and decomposing plant tissue. *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil. Under most conditions the bacterium is inactive and exists in spore form. It is not known to be pathogenic or toxic to any environmental animal or plant species. *B. pumilus* is used as a hay preservative in animal feed and in food and chemical fermentation processes. *B. pumilus* is not known to produce any toxins, although several cytolytic enzymes and antibiotic substances are well characterized. Taking these factors into consideration, no significant or irreversible adverse effects on wildlife are anticipated for the proposed soybean root treatment uses. However, any possible adverse effects to wildlife species from other than soybean seed treatment uses will have to be reevaluated if the uses of GB34 Concentrate are expanded beyond soybean seed treatment.

1. Risk Characterization for Terrestrial Wildlife

a. Avian Oral Testing Tier I, USEPA OPPTS 885.4050

Seed treatment uses are most likely to result in avian exposure. The submitted study "GB-34: An Avian Oral Pathogenicity and Toxicity Study in the Northern Bobwhite" (MRID No.452940-05) is classified as Acceptable, it is scientifically sound and fulfils the OPPTS Guideline 885.4050 testing requirement for avian hazard assessment. 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. The results of the maximum hazard dose study indicate that no avian hazard is expected from ingestion of *B. pumilus* GB-34.

b. Wild Mammal Risk Assessment Tier I, USEPA OPPTS 885.4150

Gustafson LLC has submitted a justification for a data waiver* from wild mammal toxicity/pathogenicity studies. The waiver request is based on the rationale that the active ingredient is a naturally-occurring soil, water and plant-surface colonizer, whose level in the environment will not significantly increase with the use of GB34. In addition, human health data performed on rodent species showed no detrimental effects.

B. pumilus is a ubiquitous, saprophytic, common soil microbe that contributes to nutrient cycling and displays antifungal activity. *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil . The added soil density of *B. pumilus* from the proposed seed treatment use rates represents a very small proportion of the naturally occurring bacilli in the soil and therefore is not expected to add substantially to the effects of the naturally occurring *Bacillus* populations. A series of literature searches have been conduced to determine whether any adverse effects from *B. pumilus* have been reported on mammalian species.

In addition, the mammal hazard assessment is also being performed on the basis of rodent toxicity data prepared for human health risk assessment purposes and published literature reports. The standard mammalian toxicity test data submitted to the Agency indicate no adverse effects to rodents during the acute oral and itratracheal toxicity and pathogenicity testing at the maximum hazard dose. These data show a lack of toxicity to mammals from exposure to high levels of *B. pumilus* spores. Therefore no further wild mammal testing is required.

c. Nontarget Plant Risk Assessment , Tier I, USEPA OPPTS 885.4300

Gustafson LLC. has submitted a justification for a data waiver* from terrestrial and aquatic plant toxicity/pathogenicity studies. The waiver request is based on the rationale that the active ingredient is a naturally-occurring soil, water and plant-surface colonizer, whose level in the environment will not significantly increase with the use of BB34. In addition, testing performed for efficacy evaluations showed no detrimental effects to plants. *B. pumilus* is not listed in the U.S. Department of Agriculture list of plant pathogens (Federal Plant Pest Act Regulations, 7CFR Part 330). In some studies it is found to be the most prevalent organism in the plant phylloplane. There are unconfirmed published reports of *B. pumilus* causing bacterial blotch in Balady peach in Egypt and post harvest disease of garlic cloves. The weight of evidence, however, indicates that *B. pumilus* is a normal part of the microbial flora on plants and is responsible for resistance to bacterial wilt and fungal plant diseases. *B. pumilus* is a ubiquitous, saprophytic, common soil microbe that contributes to nutrient cycling and typically occurs at 10^6 to 10^7 CFU per gram of soil . The added soil density of *B. pumilus* from the proposed seed treatment use rates represents a very small proportion of the naturally occurring bacilli in the soil and therefore is not expected to add substantially to the effects of the naturally occurring *Bacillus* populations. Therefore, hazards to plants from the proposed uses of GB34 biofungicide soybean seed treatment are not anticipated.

d. Nontarget Insect Studies, Tier I, USEPA OPPTS 885.4340

Gustafson LLC has submitted a justification for a data waiver* from aquatic and terrestrial insect toxicity/pathogenicity testing. The waiver request is based on the rationale that the active ingredient is a naturally-occurring soil, water and plant-surface colonizer, whose level in the terrestrial and aquatic environment will not significantly increase with the use of GB34, and that an extensive literature search yielded no reports of adverse effects to insect species.

1. Honey Bees

B. pumilus is commonly isolated from abdominal tissue of stingless bees and is the predominant species found in bee larval food preparations. It is one of the most commonly isolated *Bacillus* species from pollen and bee bread and has been found in the gut of worker bees. It also appears to be a common gut inhabitant of alfalfa leafcutter bees. As a result, no discernible exposure or detrimental effects to honey bees are expected from the proposed soybean seed treatment uses.

2. Other insects and other invertebrate species

Given that *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil, no adverse effects to

soil invertebrates from additional seed treatment application are expected. Also, *B. pumilus* is not listed among the known insect pathogens. As a result, no discernible detrimental effects to soil invertebrates are expected from the proposed soybean seed treatment uses. These factors are adequate to demonstrate a lack of concern for invertebrate species from the proposed soybean seed treatment uses.

2. Risk Characterization for Aquatic Wildlife

a. Freshwater Fish Testing, Tier I, USEPA OPPTS 885.4200

Gustafson LLC. has submitted a justification for a data waiver* from freshwater fish toxicity/pathogenicity studies. The request is based on the rationale that the active ingredient is a naturally occurring soil colonizer, whose level in the aquatic environment will not significantly increase from the soybean seed treatment uses. In addition, an extensive literature search yielded no reports of adverse effects to freshwater fish due to natural soil runoff of *B. pumilus*. Any *B. pumilus* that reaches aquatic environments in the form of run-off is expected to behave as it would in the wild. Therefore, freshwater fish are not expected to be exposed or harmed by *B. pumilus* GB34 as a result of the soybean seed treatment uses.

b. Freshwater Aquatic Invertebrate Testing, Tier I, USEPA OPPTS 885.4240

Gustafson LLC. has submitted a justification for a data waiver* from freshwater aquatic invertebrate toxicity/pathogenicity studies. The request is based on the rationale that the active ingredient is a naturally occurring soil colonizer, whose level in the aquatic environment will not significantly increase from the soybean seed treatment uses. *B. pumilus* occurs naturally in aquatic environments from soil run-off where *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil. Any additional exposure from the soybean seed treatment uses is extremely small, to nonexistent. In addition, in-house data on the *subtilis* group of bacilli and a lack of published reports of adverse effects show that exposure to this group of bacilli has no measurable deleterious effects on aquatic invertebrates. Therefore the proposed soybean seed treatment uses are not likely to result in adverse effects on aquatic invertebrate populations.

c. Estuarine and Marine Animal Risk Assessment, Tier I, USEPA OPPTS 885.4280.

Gustafson LLC has submitted a justification for a data waiver* from estuarine and marine animal testing. The waiver request is based on the rationale that the active ingredient is a naturally-occurring soil, water and plant surface colonizer, whose level in the estuarine/marine environment will not significantly increase from the soybean seed treatment uses. *B. pumilus* occurs naturally in aquatic environments from soil run-off where *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil. Any additional exposure from the soybean seed treatment uses is extremely small, to nonexistent. An extensive published literature search yielded no reports of adverse effects to estuarine/marine animals due to natural populations of *B. pumilus*. Therefore estuarine fish and shrimp studies are waived for soybean seed treatment uses because of very low potential for exposure.

B. Endangered species considerations

The ubiquitous occurrence of *B. pumilus* in the environment, in-house data on the *subtilis* group of bacilli and a lack of published reports of adverse effects on wildlife by *B. pumilus* indicate that exposure to this group of bacilli has no measurable deleterious effects on endangered terrestrial and aquatic species. Because BG34 biofungicide does not pose a hazard to wildlife at the proposed use rates, there also is no "may effect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

III. HAZARD ASSESSMENT

Bacillus pumilus belongs to the *subtilis* group of bacilli and is a ubiquitous bacterium commonly found in soil, water, air and decomposing plant tissue. *B. pumilus* typically occurs at 10^6 to 10^7 CFU per gram of soil. Under most conditions the bacterium is inactive and exists in spore form. It is not known to be pathogenic or toxic to any environmental animal or plant species. It is not considered to be a plant pathogen by the USDA. *B. pumilus* is used as a hay preservative in animal feed and in food and chemical fermentation processes. It is not known to produce any toxins, although several cytolytic enzymes and antibiotic substances are well characterized.

The Agency has performed a hazard assessment and determined that the soybean seed treatment uses of GB34 biofungicide will have no adverse effects to avian species, including waterfowl which is most likely to be exposed; freshwater and estuarine/marine fish and invertebrates, terrestrial and aquatic insects, including the honey bee, and terrestrial and aquatic plant species. Because BG34 biofungicide does not pose a hazard to wildlife at the proposed use rates, there also is no "may effect" finding to any endangered/threatened species listed by the U.S. Fish and Wildlife Service.

***Principles for justifying data waivers:** Pesticides Assessment Guidelines Subdivision M.

The full battery of tests for registration of MPCAs (microbial pest control agents) was designed to give basic hazard and exposure information for a microorganism with totally unknown properties. In actual practice, an MPCA is usually well identified, which may facilitate prediction of its properties and behavior. Clinical and veterinary medicine, and agricultural science, have identified most microorganisms associated with diseases (this includes birds, fish and insects). If an MPCA is taxonomically similar to a clinically or agriculturally significant microorganism, this particular area of concern should be examined closely, possibly by requiring additional testing (as provided in 40 CFR 158.75) beyond that specified in 40 CFR 158.740. Conversely, if the MPCA belongs to a group of microorganisms that have never been found in association with any disease, a case may be made for reducing, or waiving, the testing requirements for this area of concern.

40 CFR part 158 contains provisions for granting waivers for data requirements in response to specific written requests by applicants (40 CFR 158.45). OPP encourages applicants to discuss their preliminary testing plans with OPP scientists. This tailoring of the testing battery on a case-

by-case basis relies on both an accurate description of the MPCA and the existence of a reliable taxonomy for the class of microorganism to which it belongs. Some microorganisms have been closely examined than others and have a large data base from which to draw conclusions. Other microorganisms, particularly protozoa and fungi, might not be as well studied or described, and it may be difficult to predict their properties reliably from a taxonomic description. In this case, it may be more difficult to justify waiving test requirements.

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