



OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

WASHINGTON, D.C. 20460

MEMORANDUM

SUBJECT: Ecological Risk Assessment for the New Active Ingredient *Bacillus licheniformis* strain 414-01 contained in the Technical and End Use Products produced by UPL NA Inc., Proposed for FIFRA Section 3 Registration

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Executive Summary

The applicant UPL NA Inc., has submitted an application to register the technical product (100% *Bacillus licheniformis* strain 414-01) and the end use formulated products 414-02 (0.88% *B. licheniformis* 414-01) and 414-03 (33% *B. licheniformis* strain 414-01) containing the new active ingredient *Bacillus licheniformis* strain 414-01. *B. licheniformis* strain 414-01 produces secondary metabolites that inhibit the growth of nematodes. The end use products are proposed for seed treatment on corn, cotton, peanut, potato, sorghum, soybean, sugar beet, and wheat. The maximum rate for seed treatments ranges 16.3 to 195.6 mL per 100 kg of seed depending on the crop for product 414-02 and is 0.85 mL per 100 kg of seed for product 414-03 (for use on peanut and potato only). Soil applications to peanut and potato are also proposed via soil drench, chemigation, drip irrigation, or shanked-in, injected, or in-furrow applications. Foliar applications to peanut may also occur via tractor-mounted spray boom at the pegging stage, which may result in foliar exposure for peanuts. The maximum soil application rate for use on peanut and potato on the label is 5 fluid ounces of product per acre mixed in an appropriate amount of water (per the label for product 414-02). For use on peanut, when a tractor-mounted spray boom is used, 0.3 inches of water through the center pivot are used to ensure the application reaches the soil, resulting in 50 to 100 gallons of water applied per acre.

Exposure

Terrestrial animals (including birds, mammals, amphibians, reptiles, and invertebrates) and nontarget terrestrial/semi-aquatic plants may be exposed to *Bacillus licheniformis* strain 414-01 by tractor-mounted spray boom applications (peanut only) and soil applications. Birds, terrestrial-phase amphibians, reptiles, and wild mammals may be exposed when consuming soil, plants, and seeds contain or are coated with *Bacillus licheniformis* strain 414-01. The concentration that comes out of the sprayer for tractor-mounted spray applications to peanut is 3.8×10^6 CFU/mL and is the terrestrial EEC for spray applications. This concentration is relevant for foliar exposure expected for use on peanuts when banded applications from a tractor-mounted sprayer occur at pegging stage. Spray drift and runoff exposure may occur from banded applications to peanuts at pegging stage. Drift exposure will vary depending on the applied droplet size and the boom height but is expected to be no more than 5 to 45% of the maximum application rate at 5 ft off the treated field (depending on the modeled boom height and droplet size in the AgDRIFT model¹). The spray drift deposition will be no more than 1.7×10^6 CFU/mL based on 45% of applied depositing at 5 ft off the treated field if 5 fluid ounces of product are diluted in 50 gallons of water per acre. Despite the potential for off-field exposure, some degradation of *Bacillus licheniformis* strain 414-01 is expected due to ultraviolet radiation, wind, and rain, as is expected for most biological pesticides (Zhu et al., 2022).

¹ Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#atmospheric>

For the proposed uses as a seed treatment or soil-directed spray, exposure is anticipated to be low because the active ingredient will be diluted in the soil and off-field exposure via drift is expected to be minimized. The EEC for soil applications for peanut and potato will be lower than foliar estimates due to dilution of the active ingredient in the soil profile and was estimated to be 1.0×10^3 CFU/cm³.

Freshwater and estuarine/marine fish and invertebrates and aquatic plants may be exposed to *Bacillus licheniformis* strain 414-01 during intense runoff events that occur soon after application or through spray drift from foliar application on peanuts when applications occur near water bodies.

Effects Characterization

Based on laboratory testing *Bacillus licheniformis* strain 414-01 was not toxic/pathogenic to birds or mammals. Additionally, *Bacillus licheniformis* strains are not known to be plant pathogens and they are not known to be pathogens of freshwater or estuarine/marine organisms. A 13-day acute oral toxicity/pathogenicity study on honeybees (*Apis mellifera*) indicated that *Bacillus licheniformis* strain 414-01 was not toxic or pathogenic to insects. These results are consistent with the EPA's understanding of the nematocidal mode of action of *Bacillus licheniformis* strain 414-01.

Risk Characterization

Soil and Seed Applications

For soil-directed applications (e.g., soil drench, soil injection, in-furrow applications) and seed treatments, exposure is expected to be low and limited to the soil horizon on the treated. While birds and mammals may be exposed if they feed on contaminated soil or seeds, no effects will occur based on the lack of toxicity/pathogenicity in laboratory testing, thus risk is expected to be low from the proposed uses. Generally, the EPA uses bird toxicity data as a surrogate for terrestrial-phase amphibians and reptiles.² The lack of toxicity shown or expected for vertebrates in general (including birds, mammals, and fish (as discussed below)) indicate that low toxicity to terrestrial-phase amphibians and reptiles can be reasonably expected. Furthermore, *B. licheniformis* falls into the *B. subtilis* complex, and when amphibians and reptiles have been exposed to *B. subtilis* strains as a probiotic to improve gastrointestinal digestion in research studies, no adverse effects were observed (Lin et al., 2020; Rawski et al., 2016; Zhang et al., 2014). Nontarget plants will not be affected because *Bacillus licheniformis* strain 414-01 is not taxonomically related to any known plant pathogens. No effects were found when honeybees were continually exposed to the active ingredient. Additionally, due to dilution and ubiquity of *Bacillus licheniformis* strain 414-01 in the soil profile, exposure will not

² <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-0>

affect nontarget, soil dwelling invertebrates. Thus, low risk is expected for all nontarget animals and plants when exposed to the active ingredient. The rationale provided by the applicant describes that *Bacillus licheniformis* strain 414-01 is ubiquitous in soil environments (Providenti et al., 2009), and according to the applicant's label *Bacillus licheniformis* strain 414-01 is primarily toxic or pathogenic to pests such as nematodes.

Aquatic organisms may be exposed to the active ingredient, however *Bacillus licheniformis* strain 414-01 is not known to be a pathogen of freshwater or estuarine/marine organisms and the species is used as a probiotic in fish aquaculture. Additionally, concentrations of the active ingredient will not be different than background levels in these water bodies given the dilution and the predominant soil-directed and seed treatment use patterns.

Peanut Applications

Foliar Applications to Peanuts and Listed Insect Species

While risk is predicted to be low for all nontarget taxa from foliar applications due to lack of toxicity, a spatial analysis was conducted to confirm lack of exposure of listed species from the uses on peanut due to the possibility of exposure for invertebrates (particularly soft bodied invertebrates) on the treated field. Additionally, low risk is expected for terrestrial invertebrate species after soil applications and seed treatments, which is further supported by the lack of no effects in honeybees during toxicity testing. The Use Data Layer (UDL) Overlap Tool³ is a Geographic Information System tool used to identify the ranges and critical habitats of listed threatened and endangered species that may be affected by a pesticide application on a particular crop or set of crops based on spatial co-occurrence of the species and proposed use sites. The UDL Overlap Tool was used for the peanut foliar application because this is the only use pattern that has a foliar use on the label. Foliar use has a higher level of exposure for on-field nontarget invertebrates and can also drift off field during application. Therefore, the UDL Overlap Tool was used to delineate species' ranges and critical habitats to assess potential impacts to threatened and endangered nontarget terrestrial invertebrates for applications to peanuts. Overlap percentages that are <1%⁴ are not considered to be a significant overlap. The output provided a list of five insects species at 0 to 30 meters off the field that had overlap >1% but <5% with the UDL that represents use on peanuts. Furthermore, for each of the five listed insects, it is clear that the habitats that they occupy do not actually overlap with the locations of commercial peanut farming operations. While nontarget invertebrates could be exposed during foliar spray operations, given the lack of toxicity/pathogenicity to honeybees in laboratory testing and the unlikelihood of listed invertebrates being on or near the treated field, no effects are expected for nontarget terrestrial invertebrates. Additionally, nontarget

³ Available at: <https://www.epa.gov/endangered-species/provisional-models-and-tools-used-epas-pesticide-endangered-species-biological#overlap>

⁴ The overlap percentage is rounded to whole numbers due to the precision of the remotely sensed data; therefore <1% overlap represents ≤0.44% overlap, with any overlap between 0.45 and 0.99% (inclusive) rounding up to 1% overlap.

terrestrial vertebrates (including birds, mammals, amphibians, and reptiles) and plants may be exposed from foliar spray operations via contaminated food items (for animals) or spray drift and/or runoff (for plants), but these taxonomic groups will not be affected given the lack of toxicity/pathogenicity shown in the submitted studies and rationale. Furthermore, effects to the prey, pollination, habitat, and dispersal (PPHD) of listed terrestrial vertebrates and plants are not expected based on the lack of effects of the pesticide on nontarget invertebrates that these species may rely on for ecosystem services such as prey or pollination.

Conclusion

Due to lack of adverse effects in birds, mammals, nontarget plants, and nontarget insects at estimated exposure levels, expected lack of toxicity to amphibians and reptiles, and aquatic exposures that are below the concentrations that would elicit any effects, there is a reasonable expectation of no discernible effects to these taxa. The Agency therefore makes a “no effect” finding for effects to federally threatened and endangered (“listed”) birds, mammals, amphibians, reptiles, plants, terrestrial invertebrates, and aquatic organisms (animals and plants) from the proposed uses of *Bacillus licheniformis* strain 414-01.

The conclusions conveyed in this assessment were developed in full compliance with the *EPA Scientific Integrity Policy for Transparent and Objective Science*, and the *EPA Scientific Integrity Program’s Approaches for Expressing and Resolving Differing Scientific Opinions*. The full text of the *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and the EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/201402/documents/scientific_integrity_policy_2012.pdf. The full text of the *EPA Scientific Integrity Program’s approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: <https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions>.

Background

Strain 414-01 is a new isolate of *Bacillus licheniformis*. The first pesticide containing *B. licheniformis* as an active ingredient was registered by the Agency in 2003. Because *B. licheniformis* falls into the *B. subtilis* complex it is pertinent that there are 40 registered products containing *B. subtilis*, including seven manufacturing-use product(s) and 33 end-use product(s), containing 0.01% to 100% active ingredient. Seven strains of *B. amyloquefaciens* (which is also part of the *B. subtilis* complex) have been registered and are currently used as fungicidal, bactericidal, and nematocidal pesticides. None of these strains have been found to present a risk to nontarget organisms when used in accordance with their labels.

Bacillus licheniformis in general and *Bacillus licheniformis* strain 414-01 are known as ubiquitous, motile, gram-positive saprophytic soil bacteria. *Bacillus licheniformis* strain 414-01 is a new active ingredient with a manufacturing/technical use product and two end use

biopesticide products proposed for registration. *Bacillus licheniformis* strain 414-01 is a bionematicide with proposed use patterns of seed treatments and soil applications. There is also a foliar use pattern exclusively for peanuts at the pegging stage. *Bacillus licheniformis* is an industrially important bacteria that is considered part of the wider “*Bacillus subtilis* species complex” which are known to be used as biopesticides because of their antifungal properties, along with their capacity to produce secondary metabolites (Fan et al., 2017).

Due to the high similarity between *B. amyloliquefaciens*, *B. subtilis*, and *B. licheniformis* literature sources that estimate spore concentrations of *B. subtilis* have been used in this risk assessment to estimate *B. licheniformis* spore concentrations. Under most soil conditions *B. subtilis* is not biologically active but exists in the spore form at 10^6 to 10^7 CFU per gram of soil (Alexander, 1977); therefore, *B. licheniformis* is expected to be present at similar amounts. The *Bacillus licheniformis* strain 414-01 assessed in this document is proposed to control nematodes and is anticipated to have a low environmental risk profile because of its ubiquity in the natural environment, predominant seed treatment and soil-directed application methods, lack of effects on nontarget species, and lack of overlap of potential peanut use sites with the ranges and critical habitats of threatened and endangered nontarget insects.

I. Mode of Action

Bacillus licheniformis strain 414-01 produces secondary metabolites that inhibit the growth of nematodes.

II. Use and Usage

The end use products are for seed treatment on corn, cotton, peanut, potato, sorghum, soybean, sugar beet, and wheat. The maximum application rate for seed treatments ranges from 16.3 to 195.6 mL per 100 kg of seed depending on the crop for product 414-02 and is 0.85 mL per 100 kg of seed for product 414-03 (for use on peanut and potato only). Soil applications to peanut and potato can be applied via drench, in-furrow, and chemigation via drip. Additionally for peanut, foliar applications can be made at the pegging stage via tractor-mounted spray boom. For foliar applications to peanut, the 414-02 product is applied at a maximum rate of 5 liquid ounces of product in 50 gallons of diluent (water) per acre.

III. Nontarget Organism Exposure

A. Terrestrial Environments

The estimated environmental concentration (EEC) for terrestrial organisms is estimated as the concentration that comes out of the sprayer. This concentration is based on the average active ingredient content of the end use product (5.1×10^{11} CFU/g AI; average enumeration of 5

batches MRID 51662301), the quantity of product applied, and the amount this product diluted in the sprayer. To calculate the EEC, 5.1×10^{11} CFU/g AI is multiplied by 36.3 g AI/gal product⁵, which equals 1.9×10^{13} CFU/gal product. This value (1.9×10^{13} CFU/gal product) is then converted to CFU per mL to equal 4.9×10^9 CFU/mL product. Next, 4.9×10^9 CFU/mL product is converted to CFU/fl oz by multiplying by 29.6 and then multiplied by 5 fl oz/A (the maximum application rate on the label) to equal 7.2×10^{11} CFU/A. Lastly, 7.2×10^{11} CFU/A is divided by a minimum of 50 gallons of dilution water (189,271 mL) used during banded application to peanuts at pegging stage. This equals 3.8×10^6 CFU/mL spray, which is the EEC. This is the EEC for the foliar spray use on peanut at pegging stage.

The EEC for soil applications will be lower than the concentration coming out of the sprayer due to dilution of the active ingredient in the soil profile. Furthermore, for soil applications negligible drift is expected, and exposure for nontarget organisms is expected to be limited to the treated field. An acre of agricultural land, when measured to a depth of 6.7 inches, contains 6.9×10^8 cm³ of soil per acre (Penn State Ag Extension, 2016). The maximum amount of *Bacillus licheniformis* strain 414-01 applied per acre according to the proposed label is 7.2×10^{11} CFU (as explained above). This value divided by 6.9×10^8 cm³ of soil per acre yields a soil EEC of 1.0×10^3 CFU/cm³. This EEC is at least four orders of magnitude below background concentrations of *Bacillus* sp. in soil if a bulk density of 1.33 g/cm³ is assumed (10^6 to 10^7 CFU per gram of soil; Alexander, 1977); therefore, applications to soil are not expected to contribute significantly to the exposure of any nontarget organisms.

1. Birds, Mammals, Reptiles, Amphibians, and Nontarget Plants

These terrestrial species may be directly exposed to *Bacillus licheniformis* strain 414-01 during application. Birds, wild mammals, amphibians, and reptiles may also be exposed when consuming or coming into contact with soil, plants, and seeds that contain or are coated with *Bacillus licheniformis* strain 414-01 as a result of applications. Nontarget plants also may be exposed via runoff and/or spray drift.

2. Nontarget Invertebrates

For foliar applications to peanut, nontarget insects may be exposed directly or by consuming plant parts, organic/nonorganic matter, seeds, or soil. Pollinators may be exposed during or after application of the end use product and when visiting plants to gather nectar and/or pollen. Applications to the seed/soil will not significantly contribute to exposure of nontarget insects because the AI will be limited to the soil profile and such uses are not expected to increase soil concentrations above background levels. Soil dwelling terrestrial invertebrates are already exposed to *Bacillus licheniformis* and related *Bacillus* sp. at concentrations more than four orders of magnitude higher than the soil EEC (Alexander, 1977).

⁵ The label for product 414-02 states that the end use product contains a total of 0.08 lb AI per gallon of product.

B. Aquatic Environments

Freshwater and estuarine/marine fish and invertebrates and aquatic plants may be exposed to *Bacillus licheniformis* strain 414-01 during intense runoff events that occur soon after application or through spray drift when applications occur near water bodies.

Freshwater and estuarine/marine fish and invertebrates and aquatic plants may be exposed via runoff and drift. Worst-case aquatic EECs that may result from direct application to water can be estimated as follows. This concentration is based on the average active ingredient content of the end use product is 5.1×10^{11} CFU/g AI (average enumeration of 5 batches MRID 51662301), the quantity of product applied, and the amount this product is diluted for the highest application rate. To calculate the EEC, 5.1×10^{11} CFU/g AI is multiplied by 36.3 g AI/gal product⁶, which equals 1.9×10^{13} CFU/gal product. As explained above, if this value is converted to CFU/A based on the highest application rate, the amount applied is 7.2×10^{11} CFU/A. A one-acre wetland that is six inches deep contains 6.2×10^8 mL (1 acre water body at 6 inches depth has a volume of 21,780 ft³, and 1 ft³ equals 28,317 mL). Therefore, the worst-case EEC assuming direct application to water is 1.2×10^3 CFU/mL of water. This worst-case EEC which assumes direct application to water encompasses drift and runoff, because exposure from drift and runoff would be a much lower exposure scenario compared to direct water application of the active ingredient. This concentration is expected to quickly decline because *B. licheniformis* is not known to proliferate in water. Additionally, direct applications to water are not permitted on the proposed labels, so this aquatic EEC is a highly conservative estimate.

IV. Summary of Nontarget Effects Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2150 for ecological risk assessment. Data and scientific rationale were submitted to satisfy data requirements for avian oral and inhalation, wild mammal, freshwater fish, freshwater invertebrate, nontarget insect, and honeybee toxicity/pathogenicity testing.

The information provided is sufficient to satisfy the Tier I nontarget organism data requirements for ecological risk assessment for the active ingredient. Further testing of nontarget organisms at higher tiers was not required for the proposed label uses.

⁶ The label for product 414-02 states that the end use product contains a total of 0.08 lb AI per gallon of product.

Table 1. Summary of data submitted to comply with nontarget organism data requirements published in 40 CFR § 158.2150 for support of the registrations of products containing *Bacillus licheniformis* strain 414-01.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Avian Oral	885.4050	The 30-day acute oral LD ₅₀ was > 5 mL/kg-bw/day (7.2 × 10 ¹¹ CFU/mL) for Northern bobwhite (<i>Colinus virginianus</i>). No mortalities occurred. Classification: Acceptable	51662312
Avian Inhalation	885.4100	The provided rationale describes that <i>Bacillus licheniformis</i> strain 414-01 and related strains are not known to be avian pathogens. Classification: Acceptable	51662313
Wild Mammal	885.4150	The provided rationale describes two studies on rats (<i>Rattus norvegicus</i>) where no mortality, toxicity, or pathogenicity were observed. Classification: Acceptable	51662313
		An acute pulmonary toxicity/pathogenicity testing on rats (<i>Rattus norvegicus</i>) had no mortality, toxicity, or pathogenicity at 6.9 × 10 ⁹ CFU/mL. Classification: Acceptable	51662307
		An acute oral toxicity study for rats (<i>Rattus norvegicus</i>) indicated an LD ₅₀ > 5000 mg/kg-bw. No mortalities occurred. Classification: Acceptable	51662309

Data Requirement	OCSP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
Freshwater Fish	885.4200	<p>The provided rationale demonstrates that <i>Bacillus licheniformis</i> strain 414-01 is not known to be a pathogen of aquatic organisms and this bacterium is used as a probiotic in fish aquaculture. Additionally, concentrations of the active ingredient will not be different than background levels in these water bodies.</p> <p>Classification: Acceptable</p>	51662313
Freshwater Aquatic Invertebrates	885.4240	<p>The provided rationale demonstrates that <i>Bacillus licheniformis</i> strain 414-01 is not known to be a pathogen of aquatic organisms and this bacterium is used as a probiotic in fish aquaculture, indicating that pathogenicity is not expected for aquatic animals. Additionally, concentrations of the active ingredient will not be different than background levels in these water bodies.</p> <p>Classification: Acceptable</p>	51662313
Estuarine/Marine Organisms	885.4280	<p>The provided rationale demonstrates that <i>Bacillus licheniformis</i> strain 414-01 is not known to be a pathogen of marine organisms and the species is used as a probiotic in fish aquaculture. Additionally, concentrations of the active ingredient will not be different than background levels in these water bodies.</p> <p>Classification: Acceptable</p>	51662313
Nontarget Plants	885.4300	<p>The provided rationale demonstrates that <i>Bacillus licheniformis</i> strain 414-01 is not a plant pathogen and is not related to any known plant pathogens.</p> <p>Classification: Acceptable</p>	51662313
Nontarget Insects	885.4340	<p>The provided rationale describes that <i>Bacillus licheniformis</i> strain 414-01 is ubiquitous in soil environments.</p>	51662313

Data Requirement	OCSP (OPPTS) Guideline No.	Results Summary and Classification	MRID No.
		Classification: Acceptable	
Honeybee Oral Testing		<p>In a 13-day acute oral toxicity/pathogenicity study, honeybees (<i>Apis mellifera</i>) were exposed to the active ingredient continuously. The EEC is 3.8×10^6 CFU/mL (see section 3 above for terrestrial EEC calculation) and is below the No Observed Adverse Effect Concentration (NOAEC) at 6×10^8 CFU/mL. Mortality was low and no statistically significant differences compared to the controls were noted for the inactivated test substance and test groups.</p> <p>Classification: Acceptable</p>	51662314

A. Nontarget Organism Study Summaries

Study Title: Avian Oral Toxicity

MRID No.: 51662312

Classification: Acceptable

Study Summary: In a 30-day acute oral toxicity study Northern bobwhite (*Colinus virginianus*) were exposed to *Bacillus licheniformis* strain 414-01 (7.2×10^{11} CFU/mL) at a single dose volume of 5 mL/kg-bw/day by gavage for 5 consecutive days. A negative control and attenuated control (10 birds/group) were tested concurrently. No mortalities occurred in the control, attenuated control, or test substance groups. There were no significant differences in body weight or weight gain between the inactivated control and test substance groups when compared to the negative control. There were no significant differences in food consumption between the attenuated control and test substance groups when compared to the negative control group. Based on these findings, the 30-day acute oral LD₅₀ was > 5 mL/kg-bw/day (7.2×10^{11} CFU/mL).

Study Title: Honeybee Acute Oral Testing

MRID No.: 51662314

Classification: Acceptable

Study Summary: In a 13-day oral toxicity study, honeybees (*Apis mellifera*) were exposed to a negative control, an inactivated control, and the test substance *Bacillus licheniformis* strain 414-01 at three different concentrations (6×10^6 CFU/mL, 6×10^7 CFU/mL, 6×10^8 CFU/mL). No statistically significant mortality occurred in the inactivated control and the three test

substance groups compared to the negative control. Mortality at day 13 was 27% in the negative control group, 23% in the inactivated test substance group, 13% at 6×10^6 CFU/mL, 13% at 6×10^7 CFU/mL, and 17% at 6×10^8 CFU/mL. The NOAEC was determined to be 6×10^8 CFU/mL, and the test substance was not considered toxic or pathogenic to honeybees.

The remaining study requirements were all addressed by submitted rationale.

V. Literature Search Results

Because strain 414-01 is a new *B. licheniformis* strain, information in the public literature is limited on this specific strain. A literature search was conducted using the Web of Science with the terms "*Bacillus licheniformis* strain 414-01", which returned no results. A general literature search was conducted with the Web of Science using the terms "*Bacillus licheniformis*" with the respective keyword search terms "mammal," "human," "bird," "fish," "invertebrate," "honeybee," "insect," "plant," while also searching specifically by using in title "pathogen" and in title and biopesticide. The searches produced 821 results. The results primarily discussed probiotic and feed additive uses of *Bacillus licheniformis* strains for poultry feed and aquaculture, and the strains are known as common microflora present on poultry, fish, and in soil. *Bacillus licheniformis* strains have been also extensively studied for their immunoenzyme properties in cancer research, bioremediation, sewage treatment, enzymatic detergent uses, and degradation capabilities. The literature also showed that *Bacillus licheniformis* strains are known as nematocides because of their ability to produce secondary metabolites such as chitinases and proteases. The reported insecticidal activity in the literature was limited to a lab study conducted on subterranean worker termites. *Bacillus licheniformis* has also been used as a probiotic for honeybee hives and no effects against honeybees or pollinators were reported. Several scientific publications discussed the antifungal and antibacterial properties associated with a few *Bacillus licheniformis* strains. Additionally, several strains were reported to induce systemic resistance in plants.

VI. Ecological Risk Characterization

A. Terrestrial Environments

1. Birds, Mammals, Reptiles, Amphibians, and Nontarget Plants

Birds, mammals, reptiles, amphibians, and nontarget plants may be exposed as a result of the proposed applications. Concentrations on the treated field are assumed to be the concentration that will be on the soil or seeds. The EEC foliar applications is 3.8×10^6 CFU/mL spray and the soil EEC is 1.0×10^3 CFU/cm³, which is well below the dosage in acute oral 5 mL/kg-bw/day (7.2×10^{11} CFU/mL) for Northern bobwhite. The soil EEC comparison is used because cm³ are equivalent to mL, and because the density of water is 1 g/mL and soil density can range from 1.1 g/cm³ to 1.6 g/cm³. While exposure may occur, effects to birds are not

anticipated. Laboratory testing with birds did not show any effects at the maximum dose required by the guideline. *Bacillus licheniformis* strain 414-01 and related strains are not known to be avian pathogens and they are frequently found on avian feathers (MRID 51662313). *B. subtilis*, *B. amyloliquefaciens*, *B. velezensis* strains and several other strains from the *B. subtilis* complex are commonly included in bird feed as a probiotic supplement to enhance growth and digestion (Abdel-Moneim et al., 2020). Based on this information, effects to birds are not anticipated via the proposed ground/seed/foliar application use patterns.

An acute oral toxicity/pathogenicity test with rats did not show any mortality at > 5000 mg/kg-bw, and an acute pulmonary toxicity/pathogenicity testing on rats had no mortality, toxicity, or pathogenicity at 6.9×10^9 CFU/mL. No mortality occurred, and since this is a maximum challenge level effects in the field will not occur for rats. Since rats may be used as a surrogate for wild mammals, there are not any anticipated risks for wild mammals. Due to lack of effects and the maximum estimated environmental concentrations, direct and indirect effects mammals, are not anticipated when exposed to *Bacillus licheniformis* strain 414-01 via the ground/seed application use patterns.

Generally, the EPA uses bird toxicity data as a surrogate for terrestrial-phase amphibians and reptiles. The lack of toxicity shown or expected for vertebrates in general (including birds, mammals, and fish (as discussed below)) indicate that low toxicity to terrestrial-phase amphibians and reptiles can be reasonably expected. Furthermore, *B. licheniformis* falls into the *B. subtilis* complex, and when amphibians and reptiles have been exposed to *B. subtilis* strains as a probiotic to improve gastrointestinal digestion in research studies, no adverse effects were observed (Lin et al., 2020; Rawski et al, 2016; Zhang et al., 2014).

Bacillus licheniformis strain 414-01 and species from the *Bacillus subtilis* complex in general are not taxonomically related to any known plant pathogens. Additionally, a literature search did not identify any data regarding phytotoxicity caused by *Bacillus licheniformis*. Therefore, no effects to plants are anticipated as a result of application *Bacillus licheniformis* strain 414-01 via the ground/seed/foliar application use patterns.

2. Nontarget Invertebrates

The use pattern for all proposed crops is seed treatments, with soil applications (*e.g.*, soil drench, in-furrow application) also proposed for peanuts and potatoes. The soil EEC is low (1.0×10^3 CFU/cm³) and at least four orders of magnitude below background levels of *Bacillus* sp. in soil (Alexander, 1977). Although soil dwelling invertebrates may also be exposed to *Bacillus licheniformis* strain 414-01 from seed treatments and soil applications, due to dilution of the applied AI resulting in a low EEC and the ubiquity of the active ingredient and other similar microbes in the soil profile, exposure is not expected to result in effects to nontarget insects. The rationale provided by the applicant states that *Bacillus licheniformis* strain 414-01 is ubiquitous in soil environments, and according to the applicant's labels, *Bacillus licheniformis*

strain 414-01 is primarily toxic or pathogenic to pests such as nematodes. Additionally, environmental degradation of *Bacillus licheniformis* strain 414-01 is expected to further reduce potential soil exposures because of environmental factors such as ultraviolet radiation (particularly on the soil surface) and rainfall, as is expected for most biological pesticides (Zhu et. al., 2022).

In addition to soil and seed applications, foliar applications are proposed for peanuts at the pegging stage. Therefore, nontarget invertebrates may also be exposed to *Bacillus licheniformis* strain 414-01 during foliar application via banded applications with tractor-mounted spray booms. Specifically, nontarget invertebrates may be directly exposed while foraging in fields where applications are actively occurring, which is when they are likely to encounter the highest amount of *Bacillus licheniformis* strain 414-01 in their environment. As described above, the maximum EEC, the concentration that comes out of the sprayer, is 3.8×10^6 CFU/mL. Exposure could occur at this level, however effects for nontarget invertebrates are not expected because the submitted honeybee study showed that the EEC at 3.8×10^6 CFU/mL is below the No Observed Adverse Effect Concentration (NOAEC) of 6×10^8 CFU/mL. Mortality was low and no statistically significant differences were noted for the inactivated test substance and test groups compared to the negative controls. This is further supported by the overall lack of adverse effects noted in the open literature (Section 5) and by the EPA's understanding of the nematocidal mode of action of *Bacillus licheniformis* strain 414-01.

Foliar Application to Peanuts and Listed Insect Species

While risk is predicted to be low for all nontarget taxa from foliar application due to lack of toxicity, a spatial analysis was conducted to confirm lack of exposure for listed species from the uses on peanut due to the possible of exposure for invertebrates (particularly soft bodied invertebrates) on the treated field given the mode of action on nematodes. A spatial analysis was conducted for the proposed use on peanut because peanut is the only crop that has foliar use on the label. Foliar use has a higher level of exposure for on-field nontarget invertebrates and can also drift off field during application. Therefore, the EPA conducted a spatial analysis using the UDL Overlap Tool to identify the ranges and critical habitats of listed threatened and endangered species that may be affected by application to peanut based on spatial co-occurrence of the species and proposed use sites. The UDL Overlap Tool provided output that demonstrates the potential overlap (or co-occurrence) of listed terrestrial invertebrate species and use sites. The spatial analysis buffered the UDLs to 30 m off-field to cover any potential spray drift exposure for listed terrestrial invertebrates. In general, the EPA does not consider overlap percentages that are $<1\%$ ⁷ to be a significant overlap.

There are only five listed insects (*Oarisma poweshiek*, *Desmocerus californicus dimorphous*, *Hesperia dacotae*, *Elaphrus viridis*, *Icarcia icarioides fenderi*) that have $>1\%$ but $<5\%$ overlap with

⁷ The overlap percentage is rounded to whole numbers due to the precision of the remotely sensed data; therefore $<1\%$ overlap represents $\leq 0.44\%$ overlap, with any overlap between 0.45 and 0.99% (inclusive) rounding up to 1% overlap.

the UDL that represents use on peanuts (Other Row Crops UDL). Although overlap is indicated based on the UDL Overlap Tool, these species' habitats do not coincide with commercial peanut operations. The habitats of *Oarisma poweshiek*, *Hesperia dacotae*, and *Icarcia icarioides fenderi* are in Michigan, Wisconsin, Minnesota, the Dakotas, and Oregon. Peanuts are predominantly grown in Georgia, Alabama, Florida, North Carolina, South Carolina, and Texas (USDA NASS, 2022). *Elaphrus virdis* lives only on the Jepson Prairie in Solano County, California, which is a landscape dominated by prairies and vernal pools, and no peanut agriculture exists there. *Desmocerus californicus dimorphous* only lives in California's Central Valley, with the majority of historical sightings occurring in Merced, Yolo, Sacramento counties (USFWS, 2019). There is negligible commercial peanut production in California (4 acres) in Fresno and Merced counties (USDA NASS, 2022). This negligible peanut production in California is not expected to overlap substantially with either these two listed insects. The EPA expects that the overlap indicated in the output from the UDL Overlap Tool is due to other crops (e.g., hops, sugar beet, sunflowers) included in the Other Row Crops UDL in addition to peanuts, and these other crops may grow in these areas where the listed species occur. Therefore, with foliar uses on peanut, no effects are expected for listed nontarget invertebrates. While nontarget invertebrates could theoretically be exposed during foliar spray operations, given the lack of toxicity/pathogenicity to honeybees in laboratory testing and the unlikelihood of listed invertebrates being on or near the treated peanut field, no effects are expected for nontarget terrestrial invertebrates.

B. Aquatic Environments

Aquatic organisms may be exposed to *Bacillus licheniformis* strain 414-01. The worst-case EEC assuming direct application to water on a one-acre wetland would be 1.2×10^3 CFU/mL of water. *Bacillus licheniformis* strain 414-01 is not known to be a pathogen of freshwater or estuarine/marine organisms and the species is used as a probiotic in fish aquaculture. Additionally, concentrations of the active ingredient after the proposed uses are not expected to be different than background levels in these water bodies given the dilution and the predominant seed treatment and soil-directed use patterns.

VII. Listed Threatened and Endangered Species Assessment

Due to the lack of effects for birds, mammals, amphibians, reptiles, nontarget plants, nontarget invertebrates, and aquatic organisms at the EEC, and the lack of overlap and thus lack of exposure for listed nontarget invertebrates to foliar applications, there is a reasonable expectation of no discernible direct effects to these taxa. Additionally, effects to the PPHD of listed terrestrial animals and plants are not expected based on the lack of effects on nontarget invertebrates that these species may rely on for ecosystem services such as prey or pollination. The Agency therefore makes a "no effect" finding for federally listed threatened and endangered birds, mammals, amphibians, reptiles, plants, terrestrial invertebrates, and aquatic organisms (animals and plants) and their designated critical habitats from the proposed uses of *Bacillus licheniformis* strain 414-01.

VIII. References

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IX. Bibliography of Studies

MRID 51662307	Acceptable
MRID 51662309	Acceptable
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