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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

**MEMORANDUM**

**SUBJECT:** Human Health Risk Assessment of *Bacillus velezensis* strain 11604, a New Active Ingredient, in Crimson, EPA Reg. No. 98588-R (End-use Product) Proposed for Registration and an Associated Petition Requesting a Tolerance Exemption

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| **EPA Reg. No. / File Symbol:** | 98588-R (EP) |
| **Submission Nos:** | 1083866; 1083867 |
| **Action Code Nos:** | 00350013; 00350014 |
| **Active Ingredient Name:** | *Bacillus velezensis* strain 11604 |
| **PC Code:** | 11982 |
| **Tolerance Exemption Petition:** | 2F8991 |
| **MRID(s):** | 515840-01 through 515840-11<br>515840-16 through 515840-21<br>515840-27; 519795-01; 519795-02<br>520640-01 through 520640-05<br>521776-01; 522030-01; 522241-01. |
| **Applicant Name:** | BioConsortia, Inc. |

**FROM:** Joel V. Gagliardi, Ph.D.; Microbial Ecologist
Risk Assessment Branch
Biopesticides and Pollution Prevention Division

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**THROUGH:** Cassandra Kirk, Ph.D.; Senior Scientist
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**TO:** Hector Andres Maldonado; Risk Manager
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division

## I. Action Requested

Under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), BioConsortia, Inc. requests registration of an end-use product, Crimson, EPA Reg. No. 98588-R, containing the new active ingredient *Bacillus velezensis* strain 11604. This active ingredient is intended for use to control soil-borne and foliar plant diseases and is applied as a soil drench or foliar spray on a variety of fruits, vegetables, grains, bulb and root crops, ornamentals, and forage crops. Because application of the active ingredient may result in residues on food, BioConsortia, Inc. also requests establishment of a tolerance exemption for *Bacillus velezensis* strain 11604 in or on all food commodities. In support of

registration, the applicant has submitted a Confidential Statement of Formula (CSF) (dated October 11, 2023), a data matrix (dated December 05, 2022), product analysis data (MRIDs 515840-01; 515840-02; 515840-03; 515840-09; 515840-10; 515840-16; 515840-17; 519795-01; 519795-02; 519795-03; 519795-04; 519795-05; 519795-06; 519795-07; 519795-08; 519795-09; 521776-01; 522030-01; 522241-01), mammalian toxicology data (MRIDs 515840-04; 515840-05; 515840-06; 515840-07; 515840-08; 515840-11; 515840-12; 515840-013; 515840-14; 515840-15; 515840-18; 515840-19; 515840-20; 515840-21; 515840-27; 519795-01; 519795-02; 520640-01; 520640-02; 520640-03; 520640-04; 520640-05), and a tolerance exemption petition (2F8991).

## II. Executive Summary

Crimson, EPA Reg. No. 98588-R is an end-use product containing 87.05% *Bacillus velezensis* strain 11604, a new active ingredient. This bacterium is naturally occurring and found widely in soils, water, plant rhizospheres, and various foods. As a pre-harvest microbicide on food and non-food crops, its putative mode of action is to control fungi and bacteria through direct suppression and production of numerous typical metabolites and enzymes involved with nutrient cycling and microbial suppression activity.

*Bacillus velezensis* strain 11604 has no demonstrated infectivity and low acute toxicity based on the toxicity and infectivity study results and information presented for the active ingredient and its closely related species. Dietary and drinking water exposure is expected to be at the level of normal background since *Bacillus subtilis* group microbes are expected due to background levels already present in the environment and commonly consumed foods. There is potential for occupational exposure; however, no toxicological endpoints have been identified in guideline studies done at the maximum hazard dose. There are currently no residential uses proposed for the active ingredient. The Agency has determined that no further studies are needed at this time considering all the available hazard and exposure data on *Bacillus velezensis* strain 11604. FIFRA Determination: Based on the available toxicology and exposure information, no unreasonable adverse effects to humans are expected from the use *Bacillus velezensis* strain 11604 as a pesticide when EPA-approved product label instructions are followed. FFDCA Determination: Further, there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of *Bacillus velezensis* strain 11604 resulting from the proposed pesticidal uses.

## III. Background

*Bacillus velezensis* strain 11604 is a naturally occurring microorganism that has been discovered to control fungi and bacteria through direct suppression and production of numerous typical metabolites and enzymes involved with nutrient cycling and microbial suppression activity. *Bacillus velezensis* strain 11604 is naturally occurring and found widely in soils, water, plant rhizospheres, and various foods and is important in nutrient cycling, and plant protection. *Bacillus velezensis* strain 11604 exposure occurs naturally and can be found in the diet via microbes present on the surface of a variety of plant-based foods. There are currently no other pesticidal approvals or registrations for use of this microorganism. *Bacillus velezensis* strain 11604 population levels are expected to decrease to environmental background levels relatively rapidly following

application. *Bacillus velezensis* strain 11604, therefore, will not likely result in significant residues on food or in water. According to the toxicity/infectivity and acute toxicity studies, *Bacillus velezensis* strain 11604 has a low toxicity profile and no toxicological endpoints were identified.

## IV. Product Identity and Analysis Review

*Bacillus velezensis* strain 11604 is a member of the *Bacillus subtilis* group within the operational group for *Bacillus amyloliquefaciens*. Illumina sequencing (HiSeq PE150) was performed after DNA preparation using the Qiagen Powersoil DNA extraction kit. Sequencing libraries were constructed with the iGenomix RipTide kit. Illumina reads were trimmed with Q20 Trimmomatic v38, while Pacbio 10 Kb libraries were constructed by Medgenome and the prokaryotic hybrid assembler Unicycler was employed. Taxonomy was performed using GTDB-Tk v 2.1 and confirmed using the DSMZ digital Genome-to-Genome distance calculator. The final assembly was 4,079,244 base pairs in 11 contigs and was 99.2% complete; GC content was 46.3%. The closest match was *Bacillus velezensis* NRRL B-41580 followed by *Bacillus methylotrophicus* KACC 13105 and *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42. *Bacillus methylotrophicus* and *Bacillus amyloliquefaciens* subsp. *plantarum* taxonomies were determined to be later synonyms and now fall under *Bacillus velezensis* according to a provided literature review. Product characterization data was **ACCEPTABLE**.

## V. Summary of Toxicology Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2140 for *Bacillus velezensis* strain 11604 and the associated pesticide products for human health risk assessment. Scientific rationales were submitted to satisfy the generic (TGAI, technical grade of the active ingredient) data requirements for acute oral toxicity/pathogenicity and acute pulmonary toxicity/pathogenicity and the product-specific acute oral, inhalation, and dermal toxicity, and acute eye and dermal irritation data requirements for the end-use product, while studies were submitted to satisfy the remaining generic and product-specific toxicology data requirements. While there is no registered MP the applicant company submitted data on what is designated as a manufacturing-use product (MP) and indicated that the EP (end-use product) is identical so testing on the EP will suffice for these assessments. Therefore Table 1 describes information on product specific data for an MP and an EP, however, all of the data pertain to the EP. Information from the scientific rationales and studies is included in the section below, and Data Evaluation Records of the scientific rationales and studies are attached.

The information provided is sufficient to satisfy the Tier I toxicology data requirements for human health risk assessment for the active ingredient and the associated pesticide products. Further testing at higher tiers is not required for the current label uses.

**Table 1**. Summary of data submitted to comply with toxicology data requirements published in 40 CFR § 158.2140 for support of the registration of products containing *Bacillus velezensis* strain 11604.

| Data Requirement | OCSPP Guideline No. | Results Summary, Classification and Toxicity Category (As Applicable) | MRID |
|---|---|---|---|
| **Generic (TGAI) Toxicology Data** | | | |
| Acute Oral Toxicity/Pathogenicity | 885.3050 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to MRID 515840-04 (acute oral toxicity study conducted with the MP) and 515840-11 and 515840-27 (acute injection toxicity/pathogenicity study conducted with the TGAI). **Classification: Acceptable** | 515840-18 |
| Acute Pulmonary Toxicity/Pathogenicity | 885.3150 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to MRID 515840-06 (acute inhalation toxicity study conducted with the MP) and 515840-11 and 515840-27 (acute injection toxicity/pathogenicity study conducted with the TGAI). **Classification: Acceptable** | 515840-19 |
| Acute Injection Toxicity/Pathogenicity | 885.3200 | Not toxic, infective or pathogenic intravenously at 8.97 x $10^8$ CFU/rat – a pattern of clearance was established. **Classification: Acceptable** | 515840-11 515840-27 |
| Hypersensitivity Incidents | 885.3400 | The applicant reported that no hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurred during research, development, or testing of the TGAI over 7 years. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)). **Classification: Acceptable** | 515840-20 |
| Cell Culture | 885.3500 | Not required because *Bacillus velezensis* strain 11604 is not a virus (refer to test note #4 of 40 CFR § 158.2140(d)). **Classification: Acceptable** | 515840-21 |
| **Product-specific (MP) Toxicology Data –** Crimson, EPA Reg. No. 98588-R. | | | |
| Hypersensitivity Incidents | 885.3400 | The applicant reported that no hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurred during research, development, or testing of Crimson, EPA Reg. No. 98588-R. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)). **Classification: Acceptable** | 515840-20 |
| Acute Oral Toxicity | 870.1100 | Oral $LD_{50}$ Females > 5,000 mg/Kg bw. **Classification: Acceptable** **TOXICITY CATEGORY IV** | 515840-04 |
| Acute Dermal Toxicity | 870.1200 | Dermal $LD_{50}$ Combined > 5,050 mg/Kg bw. **Classification: Acceptable** **TOXICITY CATEGORY IV** | 515840-05 |
| Acute Inhalation Toxicity | 870.1300 | Inhalation $LC_{50}$ Combined > 2.07 mg/L. **Classification: Acceptable** **TOXICITY CATEGORY IV** | 515840-06 |

| Data Requirement | OCSPP Guideline No. | Results Summary, Classification and Toxicity Category (As Applicable) | MRID |
|---|---|---|---|
| Acute Eye Irritation | 870.2400 | Practically non-irritating to the eye.<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 515840-07 |
| Primary Dermal Irritation | 870.2500 | Not dermally irritating.<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 515840-08 |

| **Product-specific (EP) Toxicology Data –** Crimson, EPA Reg. No. 98588-R. | | | |
|---|---|---|---|
| Hypersensitivity Incidents | 885.3400 | The applicant reported that no hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurred during research, development, or testing of Crimson, EPA Reg. No. 98588-R. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable** | 515840-20 |
| Acute Oral Toxicity | 870.1100 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to the results of MRID No. 515840-04 (acute oral toxicity study conducted with the MP), and because this formulation contains inert ingredients that are not expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 520640-01 |
| Acute Dermal Toxicity | 870.1200 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to the results of MRID No. 515840-05 (acute dermal toxicity study conducted with the MP), and because this formulation contains inert ingredients that are not expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 520640-02 |
| Acute Inhalation Toxicity | 870.1300 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to the results of MRID No. 515840-06 (acute inhalation toxicity study conducted with the MP), and because this formulation contains inert ingredients that are not expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY III** | 520640-03 |

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| Acute Eye Irritation | 870.2400 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to the results of MRID No. 515840-07 (acute eye irritation study conducted with the MP), and because this formulation contains inert ingredients that are not expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 520640-04 |
| Primary Dermal Irritation | 870.2500 | Guideline data requirement addressed via the applicant's scientific rationale, which cited to the results of MRID No. 515840-08 (primary dermal irritation study conducted with the MP), and because this formulation contains inert ingredients that are not expected to be of toxicological concern (refer to test note #5 of 40 CFR § 158.2140(d)).<br>**Classification: Acceptable**<br>**TOXICITY CATEGORY IV** | 520640-05 |

## A. Toxicology Study Summaries

### 1. Generic (TGAI) Toxicology Data

**Study Title:** Acute Injection Toxicity and Pathogenicity – Rat (OCSPP 885.3200).
**MRID Nos.:** 515840-11; 515840-27.
**Classification: ACCEPTABLE** - not toxic, infective or pathogenic intravenously at 8.97 x $10^8$ CFU/rat – a pattern of clearance was established.
**Study Summary:** In an acute injection toxicity and pathogenicity study 15 male and 15 female ~8-9-week-old Sprague-Dawley rats were injected intravenously with *Bacillus velezensis* strain 11604 in sterile deionized water at a dose level of 8.97 x $10^8$ CFU/animal in a 0.1 mL/animal dosing volume. Five males and five females were treated with heat-inactivated *Bacillus velezensis* strain 11604 as inactive-treated controls, and an additional group of five males and five females served as untreated controls. Dosing was on Day 0, and the animals were observed for up to 64 days. Scheduled sacrifices and necropsies of 5 MPCA-treated animals/sex/day took place on Days 0, 3, 7, 14, 21, 42 and 64, with sacrifice and necropsy of the untreated and inactive-treated animals done on Day 21. The microbial enumeration method was validated in a separate study. All animals survived and appeared healthy throughout. No clinical signs were observed in animals from any group. The mean body weights and mean daily body weight gains of the male MPCA-treated animals were significantly lower than those of the controls on day 7, though as a group these animals gained weight normally over time. All other groups including female MPCA-treated animals had comparable weight gains throughout the experiment. Abnormal gross necropsy findings were noted in all groups on days 21, 42 and 64, namely; 8/10 untreated group rats had pale kidneys and 1/10 had a mottled spleen, 4/10 in the inactive test substance group had dark spleens with 2/4 having pale kidneys, 1/14 had a pale liver and 1/14 a dark spleen, in the MPCA treatment group 4/6 had pale kidneys with 2/4 having dark spleens, and 2 additional rats had dark spleens. These findings indicated a general health issue in the rat population that was not treatment related as these observations were found in both treated and untreated animals. A significant difference (lower) was noted in absolute spleen weight for

MPCA treated females, and in organ to body weight ratios (higher) for male and female brains and female spleens from MPCA treated animals. Clearance of the MPCA was demonstrated from blood and kidneys by Day 14, from the brain and cecum by Day 21, from MLN by day 42, and from lungs by day 64. At day 64 remaining *Bacillus velezensis* was detected in liver and spleen at 48-73 CFU/g, having decreased from 3.48-5.47 x $10^5$ CFU/g on Day 3 with steady progression lower over sampling days, demonstrating a pattern of clearance. *Bacillus velezensis* strain 11604 did not result in toxicity, infectivity, or pathogenicity in rats when dosed by intravenous injection with 8.97 x $10^8$ CFU/animal.

**Study Title:** Waiver requests: Acute Oral Toxicity/Pathogenicity (OCSPP 885.3050); Acute Pulmonary Toxicity/Pathogenicity (OCSPP 885.3150).
**MRID Nos.:** 520641-18; 520641-19.
**Classification: ACCEPTABLE**.
**Study Summary:** According to 40 CFR 180.2410(d)(1) the acute oral toxicity/pathogenicity study is required to support the TGAI. However, it can be combined with the unit dose portion of the acute oral toxicity study, with an EP or MP test material to fulfill the requirement for the TGAI and the MP or EP in a single study, if the new protocol is designed to address the endpoints of concern. In an acute oral toxicity study conducted at the limit dose, no toxicity was noted throughout exposure. In an intravenous injection toxicity/pathogenicity assay no toxicity, pathogenicity or infectivity was noted and clearance of the test organism was established. These two studies in combination satisfy the data required for an acute oral toxicity/pathogenicity study. For the acute pulmonary toxicity/pathogenicity data requirement no additional data on toxicity, infectivity or pathogenicity is expected if the study was conducted, however, due to the potential for inhalation when the product is applied and concerns for development of hypersensitivity to biological materials, respiratory PPE should consist of a minimum of a fitted N95 or higher rated respirator. The current label lists respiratory PPE but is not specific as to type(s) required.

**2. Product-specific (MP) Toxicology Data – Crimson, EPA Reg. No. 98588-R.**

**Study Title:** Acute Oral Toxicity - Rat (OCSPP 870.1100).
**MRID No.:** 515840-04.
**Classification: ACCEPTABLE** - Oral $LD_{50}$ Females > 5,000 mg/Kg bw.
**Toxicity Category:** IV.
**Study Summary:** In an acute oral toxicity study, three fasted female Sprague-Dawley rats were given a single oral gavage dose of BCI 11604 containing 100% *Bacillus velezensis* strain 11604 as received at a concentration of 5,000 mg/Kg body weight. The animals were observed for 14 days. All animals survived and appeared active and healthy throughout the study. All animals gained weight normally throughout the study. No observable abnormalities were found at necropsy.

**Study Title:** Acute Dermal Toxicity – Rat (OCSPP 870.1200).
**MRID No.:** 515840-05
**Classification: ACCEPTABLE** - Dermal $LD_{50}$ Combined > 5,050 mg/Kg bw.
**Toxicity Category:** IV.

**Study Summary:** In an acute dermal toxicity study, groups of five male and five female young adult Sprague-Dawley rats were dermally exposed to 5,050 mg/Kg of undiluted, well-mixed BCI 11604 containing 100% *Bacillus velezensis* strain 11604, to a clipped area comprising approximately 10% of the body surface area. Following exposure, the animals were observed for 14 days. All animals survived, gained weight, and appeared active and healthy throughout the study. Very slight erythema developed on day 1 in all animals, desquamation developed in 4 animals by day 4, with one female having severe eschar on day 4. Most symptoms of eschar and desquamation cleared by day 7 with desquamation subsiding in three remaining females by day 11; edema was not noted on any animals. No observable abnormalities were found in any animal at necropsy.

**Study Title:** Acute Inhalation Toxicity - Rat (OCSPP 870.1300).
**MRID No.:** 515840-06.
**Classification: ACCEPTABLE** - Inhalation $LC_{50}$ Combined > 2.07 mg/L.
**Toxicity Category:** IV.
**Study Summary:** In an acute inhalation toxicity study, groups of young adult Sprague-Dawley rats (5/sex/group) were exposed nose-only to BCI 11604 containing 100% *Bacillus velezensis* strain 11604 aerosolized for 4 hours at a concentration of 2.07 mg/L. The animals were observed for 14 days. All animals survived the study. All animals were reported as active and healthy during the experiment. All animals showed normal weight gain throughout the study though two females lost weight or failed to gain weight between dosing and day 7. Discolored lungs were noted at necropsy in 3/5 males and 5/5 females; otherwise, no observable abnormalities were observed.

**Study Title:** Primary Eye Irritation – Rabbit (OCSPP 870.2400).
**MRID No.:** 515840-07.
**Classification: ACCEPTABLE** – practically non-irritating to the eye.
**Toxicity Category:** IV.
**Study Summary:** In a primary eye irritation study, 0.1 mL of BCI 11604 containing 100% *Bacillus velezensis* strain 11604 as supplied was instilled into the conjunctival sac of the right eye of two male and one female New Zealand White rabbits. Animals were observed at 1, 24, 48, and 72 hours after test material instillation. Irritation was scored by the method of Draize and classified by the system of Kay and Calandra. All animals survived the study. No corneal opacity, iritis or conjunctival irritation, chemosis and/or discharge was noted on any rabbit throughout the study. Conjunctival redness was noted in 3/3 animals at 1-hour with clearance by 24 hours. The maximum average score was 2.0.

**Study Title:** Primary Dermal Irritation Study – Rabbit (OCSPP 870.2500).
**MRID No.:** 515840-08.
**Classification: ACCEPTABLE** - not dermally irritating.
**Toxicity Category:** IV.
**Study Summary:** In a primary dermal irritation study, three female New Zealand White rabbits were dermally exposed to 0.5 mL undiluted BCI 11604 containing 100% *Bacillus velezensis* strain 11604 for 4 hours on an approximately 6 $cm^2$ area of clipped body surface. The animals were observed at 1, 24, 48, and 72 hours after patch removal. Irritation was scored by the method

of Draize. No dermal erythema or edema was noted on one any animal during the study. The primary irritation index was 0.0.

### 3. Product-specific (EP) Toxicology Data – Crimson, EPA Reg. No. 98588-R.

**Study Title:** Waiver Requests: Acute Oral Toxicity (OCSPP 870.1100); Acute Dermal Toxicity (OCSPP 870.1200); Acute Inhalation Toxicity (OCSPP 870.1300); Primary Eye Irritation (OCSPP 870.2400); Primary Dermal Irritation (OCSPP 870.252).
**MRID No.:** 520641-01; 520641-02; 520641-03; 520641-04; 520641-05.
**Classification:** ACCEPTABLE
**Toxicity Category:** IV - Acute Oral and Dermal Toxicity, Eye and Dermal Irritation; III - Acute Inhalation Toxicity.
**Study Summary:** According to 40 CFR 158.2140(d)(5) waivers for any of the 870 series studies may be granted when the applicant can demonstrate the combination of inert ingredients is not likely to pose any significant health risks. The inert ingredients combined meet the existing food tolerance exemptions at 40 CFR 180.910 [Inert ingredients used pre- and post-harvest], 180.920 [Inert ingredients used pre-harvest], 180.950(e) [Minimal risk active and inert ingredients], and 180.960 [Polymers] and all ingredients are food grade. No additional toxicity, irritation, or other harmful effects are expected from addition of these ingredients in the end-use product Crimson containing 87.05% *Bacillus velezensis* strain 11604 with a combination of inert ingredients totaling 12.95% of the product. Due to the potential for inhalation when the product is applied and concerns for development of hypersensitivity to biological materials, respiratory PPE should consist of a minimum of a fitted N95 or higher rated respirator. The current label lists respiratory PPE but is not specific as to type(s) required.

## VI. Literature Search Results

*Bacillus* species are primarily soil and water associated and are commonly found in a variety of fresh produce and other foods with no ill effects from ingestion. Some sought after strains will preferentially colonize plants after application. *Bacillus* species currently registered for use as pesticides comprise about half of all microbial pesticides, with approximately half of those in the *Bacillus subtilis* group. The *Bacillus subtilis* group consists of several similar species and subspecies, currently organized as:

*Bacillus amyloliquefaciens, Bacillus siamensis, Bacillus velezensis, Bacillus atrophaeus, Bacillus inaquosorum, Bacillus licheniformis, Bacillus mojavensis, Bacillus halotolerans, Bacillus mojavensis, Bacillus paralicheniformis, Bacillus sonorensis, Bacillus spizizenii, Bacillus stercoris, Bacillus subtilis, Bacillus subtilis* subsp. *Amylosacchariticus, Bacillus subtilis* subsp. *Chungkookjang, Bacillus subtilis* subsp. *Endophyticus, Bacillus subtilis* subsp. *Globigii, Bacillus subtilis* subsp. *Krictiensis, Bacillus subtilis* subsp. *Lactipan, Bacillus subtilis* subsp. *Natto, Bacillus subtilis* subsp. *Niger, Bacillus subtilis* subsp. *Qingdao, Bacillus subtilis* subsp. *Sadata, Bacillus subtilis* subsp. *Subtilis, Bacillus tequilensis, Bacillus vallismortis*

*Bacillus subtilis* group microbes are known to produce enzymes that degrade starches (amylases are used in textile and paper production for instance), and proteins (proteases) including the

namesake subtilisin [CAS No. 9014-01-1, the majority component of commercial alcalase], that are commonly used in laundry detergents, soaps, contact lens cleaners, skin creams, cosmetics, and in food processing. *Bacillus subtilis* group secondary metabolites and enzymes were recently summarized (Harwood et al. 2018). *Bacillus subtilis* group microbes are used in food production, e.g., for various soybean fermentations (i.e., natto, tempeh), chili paste (i.e., Gochujang), and other foods either added or from naturally occurring sources (e.g., straw) and so are commonly consumed. Other strains are ingested as probiotics and have immunostimulatory activity. Direct-fed probiotics using *Bacillus subtilis* group strains are common in a variety of animal feeds and dietary supplements, including for human use (Hong et al. 2008).

A literature search [PubMed, Toxline] of the *Bacillus subtilis* group microbes using key terms such as "toxic*", "pathogen*" and "infect" indicated they are not toxic/pathogenic to humans, or associated with infections of animals, nor do they reside as normal flora in the gastrointestinal tract of animals. Being common soil microbes, *Bacillus subtilis* group strains are found in a variety of foods and in some cases may cause spoilage of improperly stored foods. Spoiled foods can cause illness due to ingestion of high levels of spoilage by-products, however these occurrences are rare in food safety references (Griffiths 2010). Overall, no additional information was gained from these searches that would alter the BPPD's understanding of the current state of the science for any potential effects of *Bacillus subtilis* group active ingredients on humans, including *Bacillus velezensis* isolates.

## VII. Human Exposure and Risk Characterization Assessment

### A. Description of Uses

This active ingredient is intended for use to control soil-borne and foliar plant diseases and is applied as a soil drench or foliar spray on a variety of fruits, vegetables, grains, bulb and root crops, ornamentals, and forage crops. Due to the potential for inhalation exposure when the product is applied at labeled rates/uses and concerns for development of hypersensitivity to biological materials, respiratory PPE should consist of a minimum of a fitted N95 or higher rated respirator. The current label lists respiratory PPE but is not specific as to type(s) required.

### B. Federal Food, Drug, and Cosmetic Act (FFDCA) Considerations

Section 408(c)(2)(A)(i) of FFDCA allows EPA to establish an exemption from the requirement of a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the exemption is "safe." Section 408(c)(2)(A)(ii) of FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings but does not include occupational exposure. Pursuant to FFDCA section 408(c)(2)(B), in establishing or maintaining in effect an exemption from the requirement of a tolerance, EPA must take into account the factors set forth in FFDCA section 408(b)(2)(C) and (D), which require EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance or tolerance exemption and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical

residue . . . .” Additionally, FFDCA section 408(b)(2)(D) requires that EPA consider “available information concerning the cumulative effects of [a particular pesticide’s] . . . residues and other substances that have a common mechanism of toxicity.”

EPA performs a number of analyses to determine the risks from aggregate exposure to pesticide residues. First, for microbial pesticides, EPA determines the pathogenicity and toxicity of the pesticide. Second, EPA examines exposure to the pesticide through food, drinking water, and other exposures that occur as a result of pesticide use in residential settings, as well as other non-occupational exposure to the substance.

### 1. Aggregate Exposure and Risk Characterization

In examining aggregate exposure, FFDCA section 408 directs EPA to consider available information concerning dietary exposures from the pesticide residue (including food and drinking water) and all other non-occupational exposures to the pesticide residue. These non-occupational exposures include exposures through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

No adverse effects of concern were observed in toxicological tests with *Bacillus velezensis* strain 11604 (described previously); therefore, the EPA did not conduct a quantitative exposure assessment.

#### a. Food Exposure and Risk Characterization

*Bacillus velezensis* and other *Bacillus subtilis* group members are naturally occurring and found widely in soils, water, plant rhizosphere, and various foods as vegetative cells and resistant spores. The spores may survive cooking but are not known to cause foodborne illness. *Bacillus velezensis* exposure already occurs naturally and can be readily found in the diet via microbes present on the surface of a variety of plant-based foods.

#### b. Drinking Water Exposure and Risk Characterization

*Bacillus velezensis* and other *Bacillus subtilis* group members are naturally occurring and found widely in soils, water, plant rhizosphere, and various foods as vegetative cells and resistant spores. The spores may survive water purification measures but are not known to cause disease or illness. *Bacillus velezensis* exposure already occurs naturally and can be readily found in surface and irrigation water sources with no known harmful effects. The application of *Bacillus velezensis* strain 11604 used as directed on the label would not be anticipated to increase exposure above background levels and/or result in any adverse effects to humans via exposure to drinking water.

#### c. Non-occupational, Residential Exposure and Risk Characterization

The label has a 4-hour restricted entry interval to treated areas, which should provide for very minimal non-occupational exposure for scenarios such as on-farm activities open to the public. The label also contains the mandatory statement “Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.” This active ingredient is intended for use to control soil-borne

and foliar plant diseases that could in general be more harmful in certain ways. There are no residential uses, and no toxic or other endpoints of concern. There is potential for non-occupational residue exposure though no toxic or other endpoints of concern were identified, and the active ingredient has a proposed food tolerance exemption and no tolerances are proposed. There are foliar applications, and potential for non-occupational exposure via spray drift. The submitted data indicate that *Bacillus velezensis* strain 11604 is a low-hazard microbe and product overall, and exposure via spray drift would not be expected to present a human health hazard.

### 2. Cumulative Effects

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, EPA consider "available information concerning the cumulative effects of [a particular pesticide's] . . . residues and other substances that have a common mechanism of toxicity."

*Bacillus velezensis* strain 11604 is not toxic and does not have a common mechanism of toxicity with other substances. Consequently, FFDCA section 408(b)(2)(D)(v) does not apply.

### 3. Determination of Safety for U.S. Population, Infants and Children

#### a. U.S. Population

For all of the reasons discussed previously, EPA concludes that there is reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of *Bacillus velezensis* strain 11604. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

#### b. Infants and Children

FFDCA section 408(b)(2)(C) provides that EPA shall apply an additional tenfold (10X) margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database on toxicity and exposure, unless EPA determines based on reliable data that a different margin of safety will be safe for infants and children. This additional margin of safety is commonly referred to as the Food Quality Protection Act Safety Factor. In applying this provision, EPA either retains the default value of 10X or uses a different additional safety factor when reliable data available to EPA support the choice of a different factor. As discussed previously, EPA has concluded that *Bacillus velezensis* strain 11604 is not toxic, pathogenic, or infective to mammals, including infants and children. Because there are no threshold levels of concern to infants, children, and adults when *Bacillus velezensis* strain 11604 is used in accordance with label directions and good agricultural practices, EPA concludes that no additional margin of safety is necessary to protect infants and children.

#### c. Occupational Exposure and Risk Characterization

No adverse effects of concern were observed in toxicological tests with *Bacillus velezensis* strain 11604 (described previously); therefore, the EPA did not conduct a quantitative exposure assessment. Due to the potential for inhalation when the product is applied and concerns for

development of hypersensitivity to biological materials, respiratory PPE consists at minimum a fitted N95 or higher rated respirator. The current label lists respiratory PPE as required.

### 4. Human Health Conclusions

EPA concludes that use of *Bacillus velezensis* strain 11604 in accordance with the proposed label will not result in unreasonable adverse effects to humans and that there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of *Bacillus velezensis* strain 11604. EPA does not expect dietary (food and drinking water) or other non-occupational risks from use of *Bacillus velezensis* strain 11604 an active ingredient in the proposed pesticide products. Data demonstrated that *Bacillus velezensis* strain 11604 is not toxic, pathogenic, irritating, or infective. Any risks resulting from exposure to individuals handling *Bacillus velezensis* strain 11604, such as sensitization resulting from repeated exposures, are expected to be minimized by use of the required personal protective equipment, including use of a respirator by mixers, loaders and applicators of this pesticide. In addition, residues of *Bacillus velezensis* strain 11604 will be covered by an exemption from the requirement of a tolerance in or on food commodities.

## VIII. References


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# Appendix I

**Table A1**. Summary of data submitted to comply with product analysis data requirements published in 40 CFR § 158.2120 for support of the registration of products containing *Bacillus velezensis* strain 11604; Confidential information has been omitted.

| Data Requirement | OCSPP Guideline No. | Results Summary and Classification (As Applicable) | MRID No. |
|---|---|---|---|
| **Product Analysis Data Crimson containing 87.05% *Bacillus velezensis* strain 11604, EPA Reg. No. 98588-R.** | | | |
| Product Identity | 885.1100 | Submitted data fulfill the requirement for product identity.<br>**Classification: Acceptable** | 519795-02 |
| Manufacturing Process | 885.1200 | Submitted data do not fulfill the requirement for manufacturing process.<br>**Classification: Acceptable** | 515840-10<br>521776-01<br>522030-01<br>522241-01 |
| Deposition of a Sample in a Nationally Recognized Culture Collection | 885.1250 | Submitted data fulfill the requirement for product identity.<br>**Classification: Acceptable** | 515840-10 |
| Discussion of Formation of Unintentional Ingredients | 885.1300 | Submitted data do not fulfill the requirement for manufacturing process.<br>**Classification: Acceptable** | 515840-10<br>522241-01 |
| Analysis of Samples | 885.1400 | Submitted data fulfill the requirement for product identity.<br>**Classification: Acceptable** | 515840-09 |
| Certification of Limits | 885.1500 | Submitted data fulfill the requirement for product identity.<br>**Classification: Acceptable** | 515640-09 |
| Color | 830.6302 | Light brown | 515840-01 |
| Physical State | 830.6303 | Liquid | 515840-01 |
| Odor | 830.6304 | Musty, grainy odor | 515840-01 |
| Stability to Normal and Elevated Temperatures, Metals, and Metal Ions | 830.6313 | Stable at for one year at 25ºC, 14 days at 54ºC | 515840-01 |
| Storage Stability | 830.6317 | Stable at for one year at 25ºC | 515840-01 |
| Miscibility | 830.6319 | Not intended to be mixed with oils or organic solvents | 515840-16 |
| Corrosion Characteristics | 830.6320 | Not corrosive to packaging material | 507345-03 |
| pH | 830.7000 | 7.5-8.5 at 25ºC | 515840-01 |
| Viscosity | 830.7100 | 400-4000 cp | 519795-01 |
| Density/Relative Density/Bulk Density (Specific Gravity) | 830.7300 | Specific gravity 1.0270 (20ºC) | 515840-03 |