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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

[redacted]

MEMORANDUM

SUBJECT: Human Health Risk Assessment of *Trichoderma atroviride* AT10, a New Active Ingredient, in the End Use Product (EP) TRICOTEN WP, Proposed for Registration and an Associated Petition Requesting a Tolerance Exemption

EPA Reg. No. / File Symbol:	96029-E (EP)
Submission No:	1092711
Action Code No(s):	402177
Active Ingredient Name:	*Trichoderma atroviride* AT10
PC Code:	119030
Tolerance Exemption Petition or Active Ingredient Tolerance Exemption:	3F9053
MRID(s):	51119701-51119714, 51119721, 51119722, 52159501
Applicant Name:	Agrotechnologias Naturales SL

FROM: Mohammed Zuber, Ph.D., Biologist
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TO: Jennifer Odom-Douglas, Risk Manager
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I. Action Requested

Under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Agrotechnologias Naturales SL, the applicant, requests registration of an end-use product (EP), TRICOTEN WP (EPA File Symbol: 96029-E), containing a new active ingredient *Trichoderma atroviride* AT10 (PC 119030). This active ingredient is intended for use as a fungicide for the

control of fungal diseases in field tomatoes, lettuce, grapevine, tomatoes in greenhouses, and for use in seed treatment for cereal grains, such as winter and spring wheat, triticale, barley, rye, etc. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. Because application of the active ingredient may result in residues on food, Agrotechnologias Naturales SL also requests establishment of a tolerance exemption for *Trichoderma atroviride* AT10 in or on all food commodities. In support of registration, the applicant has submitted a Confidential Statement of Formula (CSF) (dated April 18, 2023), data matrix (dated April 18, 2023), product analysis data (MRIDs 51119701-51119706, 52159501), mammalian toxicology data (MRIDs 51119707-51119714, 51119721, 51119722), and a tolerance exemption petition (3F9053).

II. Executive Summary

In this submission, the applicant is seeking registration of an EP TRICOTEN WP containing a new active ingredient *Trichoderma atroviride* AT10. The EP is a preventative and curative biological fungicide that is used prior to disease onset. It is comprised of 100% *Trichoderma atroviride* AT10 and byproducts (PC Code 119030) as the active ingredient with a minimum guaranteed potency of 1×10^8 colony forming units (CFUs) per gram of the product. This EP is formulated as a wettable powder and is meant for the control of fungal diseases in field tomatoes, lettuce, grapevine, tomatoes in greenhouses, and for use in seed treatment for cereal grains, such as winter and spring wheat, triticale, barley, rye, etc. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses.

Trichoderma atroviride AT10 was identified by nucleotide sequence analysis of its internal transcribed spacer (ITS) and translation elongation factor (*tef 1*) genes, and by the development of a primer pair that is unique and diagnostic to this strain. The active ingredient has no demonstrated infectivity and low acute toxicity based on the toxicity and infectivity study results and information presented for the active ingredient. Dietary and drinking water exposure is expected to be negligible and significant residues are not expected because:

(1) the EP will be applied to a limited number of crops (tomato, lettuce and grapevine);

(2) will be applied at low maximum individual and seasonal application rates. The maximum individual application rate is 1 pound of product per acre with a season maximum application rate of 3 pounds of product per acre. The application rate for the seed treatment use is 0.5 pounds of product per ton of seed;

(3) the EP will not be applied directly to water and in case of run off from field application, the active ingredient is not expected to pose a risk of exposure because of percolation through soil and municipal treatment of drinking water;

and (4) any potential residues will be low and in the event of a possible exposure, these residues will not pose a risk to human health due to lack of toxicity and pathogenicity.

There is potential for occupational exposure; however, no toxicological endpoints have been identified in the data presented from guideline studies for this active ingredient. Furthermore, use

of personal protective equipment (PPE; long sleeved shirts, long pants, shoes plus socks, water proof gloves and NIOSH-approved particulate respirators with any N, R, or P filter with NIOSH approval number prefix TC-84A, or a NIOSH-approved powered air purifying respirator with an HE filter with NIOSH approval number prefix TC-21C) as recommended by the product label will mitigate any potential for occupational exposure. There are currently no residential uses proposed for this active ingredient. The Agency has determined that no further studies are needed at this time considering all the available hazard and exposure data on *Trichoderma atroviride* AT10. Literature search conducted (on 01/08/2024) on the PUBMED search engine using the terms "*Trichoderma atroviride"* and "Human Infections" did not identify human infections due to this active ingredient. In summary, toxicology data submitted in this application demonstrated that *Trichoderma atroviride* AT10 is not toxic, pathogenic, irritating, or infective. Any risks resulting from exposure to individuals handling *Trichoderma atroviride* AT10, such as sensitization resulting from repeated exposures, are expected to be minimized by use of the required personal protective equipment. FIFRA Determination: Based on the available toxicology and exposure information, no unreasonable adverse effects to humans are expected from the use of *Trichoderma atroviride* AT10 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 *Trichoderma atroviride* AT10 resulting from the proposed pesticidal uses.

III. Background

The EP TRICOTEN WP containing a new active ingredient *Trichoderma atroviride* AT10 is used as a preventative and curative biological fungicide prior to disease onset. It is comprised of 100% *Trichoderma atroviride* AT10 and byproducts (PC Code 119030) as the active ingredient with a minimum guaranteed potency of 1×10^8 colony forming units (CFUs) per gram of the product. This EP is formulated as a wettable powder and is meant for the control of fungal diseases in field tomatoes, lettuce, grapevine, tomatoes in greenhouses, and for use in seed treatment for cereal grains, such as winter and spring wheat, triticale, barley, rye, etc. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. *Trichoderma atroviride* occurs ubiquitously in soils all over the world. The new active ingredient described in this submission *Trichoderma atroviride* AT10 was originally isolated from the soil in Tarragona, Spain. There are multiple modes of action for *Trichoderma atroviride* AT10, including competition, antibiosis, mycoparasitism, and induced resistance. Apart from antagonistic action against plant pathogens, *Trichoderma* spp. enhance plant growth and increase the uptake and availability of nutrients. *Trichoderma atroviride* AT10 was identified by nucleotide sequence analysis of its internal transcribed spacer (ITS) and translation elongation factor (*tef 1*) genes, and by the development of a primer pair that is unique and diagnostic to this strain. There are currently no other pesticidal approvals or registrations for use of this microorganism. This active ingredient has no demonstrated infectivity and low acute toxicity based on the toxicity and infectivity study results and information presented for the active ingredient. No significant residues of this active ingredient are expected on food or in water because the EP will be applied to a limited number of crops (tomato, lettuce and grapevine) at low maximum individual and seasonal application rates. Furthermore, the EP will not be applied directly to water and in case of run off from field application, the active ingredient is not expected to pose a risk of exposure because of

percolation through soil and municipal treatment of drinking water. No toxicological endpoints have been identified in the data presented from guideline toxicity studies for this active ingredient.

IV. Product Identity and Analysis Review

Executive summary of the product chemistry data for the EP TRICOTEN WP is presented below. A Data Evaluation Record (DER) for these product chemistry data is attached to this risk assessment.

Executive Summary of the Product Chemistry Data for the EP TRICOTEN WP:
TRICOTEN WP is an end-use product (EP) to be used as a biopesticide for control of fungal diseases on field tomatoes, lettuce, and grapevine, tomatoes in greenhouses, and for use as a seed treatment for cereal grains. It contains 100% w/w *Trichoderma atroviride* AT10 and byproducts, and not less than 1×10^8 CFU/g of product. There are no intentionally-added inert ingredients. The Basic CSF and product label are in agreement concerning the amount of active ingredient in the product and minimum guaranteed potency. Characterization of *Trichoderma atroviride* AT10 was adequately described. The manufacturing process was described in sufficient detail. The formation of potential impurities was fully discussed. An acceptable analysis of five batches of TRICOTEN WP was provided. The certified limits for the active ingredient given on the Basic CSF are within the OCSPP-recommended range. Determination of potency of the active ingredient involved a CFU count. The data reported for the Group B 830 series guidelines are satisfactory. The product chemistry data for TRICOTEN WP are classified as acceptable.
CLASSIFICATION: ACCEPTABLE

V. Summary of Toxicology Data

Table 1 provides the status of the data requirements as published in 40 CFR § 158.2140 for the active ingredient *Trichoderma atroviride* AT10 and the end -use product TRICOTEN WP for human health risk assessment. Scientific rationale in lieu of data was submitted to satisfy the acute oral toxicity data requirement for the active ingredient *Trichoderma atroviride* AT10 and for the EP TRICOTEN WP. The Agency determined that the submitted scientific rationale in lieu of data was acceptable. Studies were submitted to satisfy the remaining toxicology data requirements for the active ingredient *Trichoderma atroviride* AT10 and for the EP TRICOTEN WP. Information from the scientific rationale and studies is included in the section below, and Data Evaluation Records of the scientific rationale 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 product. 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 an end use product TRICOTEN WP containing the microbial active ingredient *Trichoderma atroviride* AT10.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary, Classification and Toxicity Category (As Applicable)	MRID
Toxicology Data for the microbial active ingredient *Trichoderma atroviride* AT10			
Acute Oral Toxicity/Pathogenicity	885.3050	*Trichoderma atroviride* AT10 does not appear to be toxic, pathogenic, or infective in rats when administered by the oral route at a dose $\geq 10^8$ CFU/animal. Mortality, clinical signs, absolute body weights and body weight gains, and gross pathology were not adversely affected by treatment. Clearance of the active ingredient was demonstrated in the lungs, blood, brain, liver, spleen, kidney, lymph node or fecal samples. **Classification: Acceptable**	51119707
Acute Pulmonary Toxicity/Pathogenicity	885.3150	The MPCA was not toxic, infective or pathogenic in rats exposed to 10^7 CFU/animal via intratracheal route. There were no adverse effects on mortality, clinical signs, or gross pathology. No signs of infectivity were observed in kidneys, brain, liver, lungs, spleen, blood and mesenteric lymph nodes. Complete clearance of the MPCA was demonstrated after day 14 but before day 21 in all test animals. Thus, a pattern of clearance for the MPCA was demonstrated in this study. Because of the use of lower than Agency required dosing for the test substance, this acute pulmonary infectivity and toxicity study is classified as **Supplemental.** The results reported in this study are useful to determine hazard or the lack thereof for this active ingredient. **Classification: Supplemental**	51119708 51119721
Acute Injection Toxicity/Pathogenicity	885.3200	There were no mortalities, abnormal clinical signs, or abnormal gross necropsy findings, or adverse effects on body weights of rats treated with 2 x 10^7 CFU/animal of *Trichoderma atroviride* AT10 via intraperitoneal injection. Thus, no toxicity, infectivity, or pathogenicity were reported in test animals. However, the Agency notes that this is not a clearance study. This study was conducted in accordance with the guideline recommendations for an acute injection toxicity and pathogenicity study (OCSPP 885.3200) in the rat. **Classification: Acceptable**	51119709
Product-specific (EP) Toxicology Data – TRICOTEN WP (EPA File Symbol: 96029-E)			
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 TRICOTEN WP. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)). **Classification: Acceptable**	51119722
Acute Oral Toxicity	870.1100	Testing waived based on an **acceptable oral toxicity/pathogenicity study** with the active ingredient, which is identical in composition to the EP. **Classification: Acceptable** **TOXICITY CATEGORY III**	51119710 51119707

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary, Classification and Toxicity Category (As Applicable)	MRID
Acute Dermal Toxicity	870.1200	Dermal LD_{50} (male rats, female rats, and male and female rats combined) > 2000 mg/kg. **Classification: Acceptable** **TOXICITY CATEGORY III**	51119711
Acute Inhalation Toxicity	870.1300	Inhalation LC_{50} (male rats, female rats, and male and female rats combined) > 2.46 mg/L **Classification: Acceptable** **TOXICITY CATEGORY IV**	51119712
Acute Eye Irritation	870.2400	TRICOTEN WP was non-irritating to the eyes of rabbits. Conjunctival irritant effects seen initially one hour post treatment were fully reversible within 24 hours. No corneal effects were observed in this study. **Classification: Acceptable** **TOXICITY CATEGORY IV**	51119713
Primary Dermal Irritation	870.2500	TRICOTEN WP is not a dermal irritant. No erythema, no oedema and no clinical signs of systemic toxicity were observed in the treated animals. **Classification: Acceptable** **TOXICITY CATEGORY IV**	51119714

A. Toxicology Study Summaries

1. Generic Toxicology Data

The active ingredient *Trichoderma atroviride* AT10 and the EP TRICOTEN WP are identical in composition. Therefore, the data summarized below are applicable to the active ingredient per se and also the EP.

2. Product-specific Toxicology Data – TRICOTEN WP (EPA File Symbol: 96029-E)

Study Title: Acute Oral Toxicity/Pathogenicity
MRID No.: 51119707
Classification: Acceptable
Toxicity Category: Not Applicable
Study Summary: In an acute oral toxicity/pathogenicity study (MRID 51119707), 12 male and 12 female Wistar rats were given single oral doses of *Trichoderma atroviride* AT10 WP (*Trichoderma atroviride* AT10; 5×10^8 CFU/g) in physiological saline at a dose level of 6.30 x 10^8 CFU/animal in males or 8.15×10^8 CFU/animal in females. Additional groups of animals were untreated non-shelf controls (2/sex), untreated shelf controls (2/sex), and inactive-treated animals (3/sex) that were treated with the inactivated test article. Treatment was on Day 0 and the animals were observed for up to 21 days. Interim sacrifices of active-treated animals (3 animals/sex/day) were done on Days 3, 7, and 14, and terminal sacrifice of the remaining active-treated animals (3/sex), the inactive treated animals, and all untreated controls was done on Day 21. Data from the untreated non-shelf and shelf controls were combined for the purpose of statistical analysis.

Mortality, clinical signs, absolute body weights and body weight gains, and gross pathology were not adversely affected by treatment. Viable test organism was detected in all six of the fecal samples taken from active-treated animals on Day 1 at mean counts of 2.4×10^5 CFU/g and 5.45×10^5 CFU/g for males and females, respectively. No viable spores were detected in fecal samples collected from active-treated animals on Days 3, 7, 14, or 21 or in any of the blood, brain, liver, spleen, kidney, or lymph node samples taken from active-treated animals. No CFUs were detected in the samples of feces taken from inactive-treated animals (Days 0 and 21) or in the blood/tissue samples taken from untreated animals or inactive-treated animals on Day 21. Viable test organism was found in 2/6 lung samples taken from active-treated animals killed on Day 3 (at 2.1×10^4 CFU/g and 5.8×10^4 CFU/g) and was not found in any additional lung samples taken thereafter. The presence of MPCA in the lungs was most likely related to aspiration of the test material during dosing rather than systemic spread of the organism.

Based on the results of this study, *Trichoderma atroviride* AT10 does not appear to be toxic, pathogenic, or infective in rats when administered by the oral route at a dose $\geq 10^8$ CFU/animal. The study author demonstrated clearance of the active ingredient in this study.

This acute oral toxicity and pathogenicity study is classified as **Acceptable**. The study was conducted in accordance with the guideline recommendations for an acute oral toxicity and pathogenicity study in the rat (OCSPP 885.3050; OECD IIM 5.3.2).
CLASSIFICATION: ACCEPTABLE

Study Title: Acute Pulmonary Toxicity/Pathogenicity
MRID No.: 51119708, 51119721
Classification: Supplemental
Toxicity Category: Not Applicable
Study Summary: In an acute pulmonary toxicity and pathogenicity study (MRID 51119708), 17 male and 17 female Wistar rats were exposed by the intratracheal route to *Trichoderma atroviride* AT10 WP (*Trichoderma atroviride* AT10; 5×10^8 CFU/g) in physiological saline at dose levels of 1.13×10^7 CFU/animal (males) or 0.91×10^7 CFU/animal (females). The animals were treated on Day 0, then observed for up to 21 days with interim sacrifices (of 3 animals/sex) on Day 0 and Days 3, 7, and 14. Three males and three females were treated with heat-inactivated test item as inactive-treated controls, and an additional group of two males and two females served as untreated controls. Terminal sacrifice of active-treated animals (5/sex) and all controls was done on Day 22. The administered dose was stated to be the maximum that could be attained. Additional supporting information was provided in a separate volume (MRID 51119721) in support of the dosage for the test substance used in this study. The Agency determined this to be satisfactory and does not require the applicant to repeat this study at the guideline required dosing of 1×10^8 CFU/animal.

There were no adverse effects on mortality, clinical signs, or gross pathology. No signs of infectivity were observed in kidneys, brain, liver, lungs, spleen, blood, and mesenteric lymph nodes. Complete clearance of the MPCA was demonstrated after day 14 but before day 21 in all test animals. The applicant, thus, demonstrated a pattern of clearance for the MPCA in this study. Therefore, under the conditions of this study, the MPCA was not toxic or pathogenic in test animals.

Because of the use of lower than Agency required dosing for the test substance, this acute pulmonary infectivity and toxicity study is classified as **Supplemental.** The results reported in this study are useful to determine hazard or the lack thereof for this active ingredient.
CLASSIFICATION: SUPPLEMENTAL

Study Title: Acute Injection Toxicity/Pathogenicity
MRID No.: 51119709
Classification: Acceptable
Toxicity Category: Not Applicable
Study Summary: In an acute injection toxicity and pathogenicity study (MRID 51119709), 3 male and 3 female 10-week-old Wistar rats were given intraperitoneal injections of *Trichoderma atroviride* AT10 WP (*Trichoderma atroviride* AT10; 5×10^8 CFU/g) in physiological saline at dose levels of 2.6 x 10^7 CFU/animal in males or 2.1 x 10^7 CFU/animal in females. Three additional groups were given intraperitoneal injections, as follows: 2 animals/sex were treated with physiological saline (the vehicle); 2 animals/sex were treated with "Reference Material" (an inert ingredient of the test item) in physiological saline at the concentration that would occur in the active-test-item formulation; and 3 animals/sex were treated with inactivated test item in physiological saline. Treatment was on Day 0, and the animals were observed for up to 21 days.

There were no mortalities, abnormal clinical signs, or abnormal gross necropsy findings, and treatment with active test item, inactivated test item, or the inert ingredient did not adversely affect body weight. In this study, *Trichoderma atroviride* AT10 WP did not result in toxicity, infectivity, or pathogenicity in rats when dosed by intraperitoneal injection at a dose of 2.1 to 2.6 x 10^7 CFU/animal. However, the Agency notes that this is not a clearance study.

This acute injection toxicity and pathogenicity study is classified as **Acceptable**. This study was conducted in accordance with the guideline recommendations for an acute injection toxicity and pathogenicity study (OCSPP 885.3200) in the rat.
CLASSIFICATION: ACCEPTABLE

Study Title: Acute Oral Toxicity
MRID No.: 51119710
Classification: Acceptable
Toxicity Category: III
Study Summary: The manufacturing use product (MUP) *Trichoderma atroviride* AT10 and the end use product (EP), TRICOTEN WP, are identical in composition and contain a minimum active ingredient concentration of 1 x 10^8 CFU/gram. The applicant is relying on the results of the acute oral toxicity/pathogenicity study (885.3050; MRID 51119707) conducted with *T. atroviride* AT10 to satisfy the acute oral toxicity data requirement for these products. In this acute oral toxicity/pathogenicity study, the animals were dosed with the active ingredient via oral administration - 6.30×10^8 CFU/animal (males) and 8.15×10^8 CFU/animal (females). This administered dose corresponds to 3636 mg/kg in males and 5118 mg/kg in females. No mortality and no toxicity/pathogenicity effects were noted in this study over a period of 21 days. Therefore, it is highly unlikely that mortality and any adverse effects would be observed in an acute oral toxicity study. Based on the active ingredient dose levels used in this study, it can be calculated that the LD_{50} would fall between Toxicity Categories III and IV. The applicant would

like to assign Toxicity Category of III for this active ingredient in an acute oral toxicity study.
CLASSIFICATION: ACCEPTABLE, Toxicity Category: III

Study Title: Acute Dermal Toxicity
MRID No.: 51119711
Classification: Acceptable
Toxicity Category: III
Study Summary: In an acute dermal toxicity study (MRID 51119711), groups (5/sex) of young adult Wistar rats obtained from Charles River Laboratories with a body weight ranging between 227 and 263 grams were dermally exposed to *Trichoderma atroviride* AT10 WP (containing 10% active ingredient) in water to 10% of body surface area at a dose of 2000 mg/kg bw. Test sites were covered with a semi-occlusive dressing for 24 hours. Animals were then observed for 14 days.

Dermal LD_{50} Males = >2000 mg/kg bw
Females = >2000 mg/kg bw
Combined = >2000 mg/kg bw

No mortality, no adverse clinical signs and no skin irritation were observed throughout the study. EPA Toxicity Category III. This acute dermal study is classified acceptable. It does satisfy the guideline requirement for an acute dermal study (OPPTS 870.1200; OECD 402) in the rat.
CLASSIFICATION: ACCEPTABLE, Toxicity Category: III

Study Title: Acute Inhalation Toxicity
MRID No.: 51119712
Classification: Acceptable
Toxicity Category: IV
Study Summary: In an acute inhalation toxicity study (MRID 51119712), groups of eight week old (5/sex) of Wistar rats obtained from Charles River Laboratories (with body weights ranging between 326-338g and 204-222g, for males and females, respectively) were exposed (nose only) via the inhalation route to *Trichoderma atroviride* AT10 WP containing 10% active ingredient (as an aerosolized powder) for 4 hours at the maximum achievable concentrations of 2.46 mg/L. Animals were then observed for 14 days.

LC_{50} Males = >2.46 mg/L
LC_{50} Females = >2.46 mg/L
LC_{50} Combined = >2.46 mg/L

The basis for toxicity category determination was lack of deaths in both male and female rats at the maximum achievable concentrations of 2.46 mg/L. EPA Toxicity Category IV. This acute inhalation study is classified as acceptable. It does satisfy the guideline requirement for an acute inhalation study (OPPTS 870.1300) in the rat.
CLASSIFICATION: ACCEPTABLE, Toxicity Category: IV

Study Title: Primary Eye Irritation
MRID No.: 51119713
Classification: Acceptable

Toxicity Category: IV
Study Summary: In a primary eye irritation study MRID 51119713, a single dose of 0.1g of the test material *Trichoderma atroviride* AT10 WP was instilled into the conjunctival sac of the left eye of three young adult (10-week-old) New Zealand white male rabbits (obtained from S&K-LAP Kft. in Hungary with a body weight ranging between 2813 and 3038 g) for 1 hour. The treated eyes were rinsed with physiological saline solution one hour post administration of the test material. Scoring of irritation was performed at 1, 24, 48 and 72 hours post application of the test material by the method of Draize (1977).

In this study, the test material *Trichoderma atroviride* AT10 WP is not considered an eye irritant. Conjunctival irritant effects seen initially one hour post treatment were fully reversible within 24 hours. No corneal effects were observed in this study. Therefore, the test substance belongs to EPA Toxicity Category IV. This study is classified as acceptable. It does satisfy the guideline requirement for a primary eye irritation study (OPPTS 870.2400) in the rabbit.
CLASSIFICATION: ACCEPTABLE, Toxicity Category: IV

Study Title: Primary Dermal Irritation
MRID No.: 51119714
Classification: Acceptable
Toxicity Category: IV
Study Summary: In an acute dermal irritation study (MRID 51119714), three young adult (10-week-old) New Zealand white male rabbits (obtained from S&K-Lap Kft. in Hungary with a body weight ranging between 2876 and 3049 g) were dermally exposed to 0.5 g of the test substance moistened with water for 4 hours. Test sites, intact shaved flanks, were approximately 6 cm^2 and were covered with a semi-occlusive dressing. The primary irritation index (P.I.I.) was scored at 1, 24, 48 and 72 hours after the removal of the dressing, following the method of Draize et al. (1944).

In this study, *Trichoderma atroviride* AT10 WP is not a dermal irritant. The primary irritation index was calculated to be 0.00. No erythema, no oedema and no clinical signs of systemic toxicity were observed in the treated animals throughout the study. The test substance belongs to EPA Toxicity Category IV. This study is classified as acceptable. It does satisfy the guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.
CLASSIFICATION: ACCEPTABLE, Toxicity Category: IV

Study Title: Hypersensitivity Incidents
MRID No.: 51119722
Classification: Acceptable
Toxicity Category: Not Applicable
Study Summary: 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 TRICOTEN WP. Any future hypersensitivity incidents must be reported to the EPA (refer to test note #3 of 40 CFR § 158.2140(d)).
CLASSIFICATION: ACCEPTABLE

VI. Human Exposure and Risk Characterization Assessment

A. Description of Uses

In this submission, the applicant is seeking registration of an EP TRICOTEN WP (96029-E) containing a new active ingredient *Trichoderma atroviride* AT10 (PC 119030). The EP is a preventative and curative biological fungicide that is used prior to disease onset. It is comprised of 100% *Trichoderma atroviride* AT10 and byproducts (PC Code 119030) as the active ingredient with a minimum guaranteed potency of 1 x 10^8 colony forming units (CFUs) per gram of the product. This EP is formulated as a wettable powder and is meant for the control of fungal diseases in field tomatoes, lettuce, grapevine, tomatoes in greenhouses, and for use in seed treatment for cereal grains, such as winter and spring wheat, triticale, barley, rye, etc. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. The EP TRICOTEN WP will be applied at low maximum individual and seasonal application rates. The maximum individual application rate is 1 pound of product per acre with a season maximum application rate of 3 pounds of product per acre. The application rate for the seed treatment use is 0.5 pounds of product per ton of seed.

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 *Trichoderma atroviride* AT10 (Table1); therefore, the EPA did not conduct a quantitative exposure assessment. At this point, the Agency would like to reiterate that the EP TRICOTEN WP contains the microbial active ingredient *Trichoderma atroviride* AT10 at a minimum guaranteed potency of 1×10^8 colony forming units (CFUs) per gram of the product. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. This EP will be applied at low maximum individual and seasonal application rates. Therefore, the pesticidal use of this EP is not expected to significantly increase the levels of *Trichoderma atroviride* AT10 naturally present in the treated environment (*Trichoderma atroviride* occurs ubiquitously in soils all over the world.). Furthermore, since food crops undergo a post-harvest washing process to remove soil and surface residues, it is unlikely that significant amounts of this active ingredient would remain on treated crops. Should this active ingredient remain on food commodities, the results of the guideline mammalian toxicology testing performed with *Trichoderma atroviride* AT10 demonstrated a lack of adverse effects (toxicity, pathogenicity or infectivity). Thus, the Agency concludes that no toxicity, pathogenicity, or infectivity is likely to occur with this type of exposure resulting from the use of this microbial pesticide when applied in accordance with label directions and good agricultural practices. Therefore, exposure to any residues of *T. atroviride* AT10 from food commodities should not be of concern for human health.

The active ingredient *T. atroviride* AT10 will not be applied directly to water. Runoff from field application is a possibility; however, percolation through soil would reduce the possibility of exposure to this active ingredient via drinking water. Furthermore, if this active ingredient was to enter drinking water systems, it will be subjected to standard drinking water treatment processes and thereby reducing the possibility of its exposure via drinking water. Should *T. atroviride* AT10 be present in water, supporting toxicological data and information indicate that no toxicity, pathogenicity, or infectivity is likely to occur with this type of exposure resulting from the use of this microbial pesticide when applied in accordance with label directions and good agricultural practices. Therefore, exposure to any residues of *T. atroviride* AT10 from drinking water should not be of concern for human health.

The Agency notes that no residential uses are proposed for the EP TRICTOTEN WP containing the active ingredient *Trichoderma atroviride* AT10. In addition, the Agency has registered products containing *Trichoderma atroviride* strain SC1 and issued an exemption from the requirement of tolerance in or on all food commodities (40 CFR 180.1378). *Trichoderma atroviride* AT10, the active ingredient under consideration for registration in this application, shares significant nucleotide sequence similarity with the previously registered *Trichoderma atroviride* strain SC1 (results presented in Confidential Appendix). Furthermore, *Trichoderma atroviride* AT10 also shares significant sequence similarities with *Trichoderma harzianum,*

Trichoderma asperellum and *Trichoderma virens* (results presented in Confidential Appendix). The Agency has previously registered products containing *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* and issued respective tolerance exemptions. Taken together, as a result of these exposure and hazard findings, and in light of the discussion presented above, no negative effects on human health are expected from exposure to *Trichoderma atroviride* AT10 through food or drinking water.

a. Food Exposure and Risk Characterization

No adverse effects of concern were observed in toxicological tests with *Trichoderma atroviride* AT10 (Table1); therefore, the EPA did not conduct a quantitative exposure assessment. The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. This EP will be applied at low maximum individual and seasonal application rates. Therefore, the pesticidal use of this EP is not expected to significantly increase the levels of *Trichoderma atroviride* AT10 naturally present in the treated environment (*Trichoderma atroviride* occurs ubiquitously in soils all over the world.). Furthermore, since food crops undergo a post-harvest washing process to remove soil and surface residues, it is unlikely that significant amounts of this active ingredient would remain on treated crops. Should this active ingredient remain on food commodities, the results of the guideline mammalian toxicology testing performed with *Trichoderma atroviride* AT10 demonstrated a lack of adverse effects (toxicity, pathogenicity or infectivity). Thus, the Agency concludes that no toxicity, pathogenicity, or infectivity is likely to occur with this type of exposure resulting from the use of this microbial pesticide when applied in accordance with label directions and good agricultural practices. Therefore, exposure to any residues of *T. atroviride* AT10 from food commodities should not be of concern for human health.

As noted above, *Trichoderma atroviride* AT10, the active ingredient under consideration for registration in this application, shares significant nucleotide sequence similarity with the previously registered *Trichoderma atroviride* strain SC1 (40 CFR 180.1378; results presented in Confidential Appendix). Furthermore, *Trichoderma atroviride* AT10 also shares significant sequence similarities with *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* (results presented in Confidential Appendix). The Agency has previously registered products containing *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* and issued respective tolerance exemptions. Taken together, as a result of these exposure and hazard findings, and in light of the discussion presented above, quantitative food exposure assessment was not performed for *Trichoderma atroviride* AT10.

b. Drinking Water Exposure and Risk Characterization

The active ingredient *T. atroviride* AT10 will not be applied directly to water. Runoff from field application is a possibility; however, percolation through soil would reduce the possibility of exposure to this active ingredient via drinking water. Furthermore, if this active ingredient was to enter drinking water systems, it will be subjected to standard drinking water treatment processes and thereby reducing the possibility of its exposure via drinking water. Should *T. atroviride* AT10 be present in water, supporting toxicological data and information indicate that

no toxicity, pathogenicity, or infectivity is likely to occur with this type of exposure resulting from the use of this microbial pesticide when applied in accordance with label directions and good agricultural practices. Therefore, exposure to any residues of *T. atroviride* AT10 from drinking water should not be of concern for human health.

b. Non-occupational, Residential Exposure and Risk Characterization

The EP TRICTOTEN WP containing the active ingredient *Trichoderma atroviride* AT10 is intended for agricultural use only. It will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. This EP will be applied at low maximum individual and seasonal application rates. Application of the EP to tomatoes in greenhouses and to field grapevine via foliar spray (ground) reduces the likelihood of off-site, airborne movement of this active ingredient. Non-occupational exposure through drift is possible for application of the EP to field tomatoes and lettuce via drip irrigation. However, due to the relatively low application rates proposed for this EP, and due to the fact that no residential uses are proposed, the likelihood of residential exposure to this microbial active ingredient is low. Should residential or non-occupational exposures occur, the results of the guideline mammalian toxicology testing performed with this active ingredient demonstrated a lack of adverse effects (toxicity, pathogenicity or infectivity). Therefore, non-occupational, residential exposure to any residues of *T. atroviride* AT10 should not be of concern for human health, when this microbial pesticide is used in accordance with label directions and good agricultural practices.

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."

Trichoderma atroviride AT10 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 *Trichoderma atroviride* AT10. 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 *Trichoderma atroviride* AT10 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 *Trichoderma atroviride* AT10 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

The EP will be applied via foliar spray (ground) to field grapevine, and via drip irrigation to field tomatoes and lettuce, and to tomatoes in greenhouses. This EP will be applied at low maximum individual and seasonal application rates. The maximum individual application rate is 1 pound of product per acre with a season maximum application rate of 3 pounds of product per acre. Therefore, the pesticidal use of this EP is not expected to significantly increase the levels of *Trichoderma atroviride* AT10 naturally present in the treated environment (*Trichoderma atroviride* occurs ubiquitously in soils all over the world). However, there is a potential for occupational exposure to applicators and other handlers through the dermal, ocular, and inhalation routes. The Agency notes that the supporting mammalian toxicological testing has shown a lack of irritation, toxicity, infectivity, or pathogenicity associated with this active ingredient. Furthermore, the product label for the EP TRICOTEN WP requires that the applicators and other handlers wear appropriate personal protective equipment (PPE): long sleeved shirts, long pants, shoes plus socks, water proof gloves and NIOSH-approved particulate respirators with any N, R, or P filter with NIOSH approval number prefix TC-84A, or a NIOSH-approved powered air purifying respirator with an HE filter with NIOSH approval number prefix TC-21C. Provided that the mandatory use of the PPE identified on the product label for the EP TRICOTEN WP is strictly followed by the applicators and other handlers, occupational exposure to Trichoderma atroviride AT10 is not expected to exceed any toxicity thresholds.

No adverse effects of concern were observed in toxicological tests with *Trichoderma atroviride* AT10, as described previously; therefore, the EPA did not conduct a quantitative exposure assessment.

3. Human Health Conclusions

EPA concludes that use of *Trichoderma atroviride* AT10 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 *Trichoderma atroviride* AT10. EPA does not expect dietary (food and drinking water) or other non-occupational risks from use of *Trichoderma atroviride* AT10 as an active ingredient in the proposed pesticide products. Data demonstrated that *Trichoderma atroviride* AT10 is not toxic,

pathogenic, irritating, or infective. Any risks resulting from exposure to individuals handling *Trichoderma atroviride* AT10, such as sensitization resulting from repeated exposures, are expected to be minimized by use of the required personal protective equipment. In addition, residues of *Trichoderma atroviride* AT10 will be covered by an exemption from the requirement of a tolerance in or on food commodities.

As noted above, *Trichoderma atroviride* AT10, the active ingredient under consideration for registration in this application, shares significant nucleotide sequence similarity with the previously registered *Trichoderma atroviride* strain SC1 (40 CFR 180.1378; results presented in Confidential Appendix). Furthermore, *Trichoderma atroviride* AT10 also shares significant sequence similarities with *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* (results presented in Confidential Appendix). The Agency has previously registered products containing *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma virens* and issued respective tolerance exemptions.

VII. Literature Search Findings

On 01/08/2024, the Agency conducted a literature search on the PUBMED search engine using the terms "*Trichoderma atroviride*" and "Human Infections". This search yielded only 7 publications, and none of these publications reported human infections. To the contrary, Saravanakumar et al. (2019) reported positive effects mediated by *Trichoderma atroviride* in protecting human health. These investigators identified TM 2 (4H-1,3-dioxin-4-one-2,3,6-trimethyl), a metabolite produced by *Trichoderma atroviride,* using high-performance liquid chromatography (HPLC) and ^{1}H and ^{13}C nuclear magnetic resonance spectrometer (NMR), and demonstrated that TM2-treated human prostate cancer cells displayed higher reactive oxygen species (ROS), cell death and apoptosis-related protein expression. TM2 also induced significant inhibition of Multi-Drug Resistant (MDR) *Helicobacter pylori* and Shigella toxin producing *Escherichia coli* (STEC) as evident by in vitro and microscopic observations of bacterial cell death. In dual-culture in vitro bioassays *Trichoderma atroviride* showed complete growth inhibition of *Aspergillus niger* and *A. fumigatus*, within 3 to 7 days. Aspergillus strongly affects health of humans, animals, and plants worldwide. Also, volatile compounds (VOCs) of *T. atroviride* exhibited 33-100% growth inhibition of *A. niger*, within 3 days. (Erfandoust et al., 2020).

VIII. References

Erfandoust R, Habibipour R, Soltani J. Antifungal activity of endophytic fungi from Cupressaceae against human pathogenic Aspergillus fumigatus and Aspergillus niger. J Mycol Med. 2020 Sep;30(3):100987. doi: 10.1016/j.mycmed.2020.100987. Epub 2020 May 8. PMID: 32499133.

Saravanakumar K, Mandava S, Chellia R, Jeevithan E, Babu Yelamanchi RS, Mandava D, Wen-Hui W, Lee J, Oh DH, Kathiresan K, Wang MH. Novel metabolites from Trichoderma atroviride against human prostate cancer cells and their inhibitory effect on Helicobacter pylori and Shigella toxin producing Escherichia coli. Microb Pathog. 2019 Jan; 126:19-26. doi: 10.1016/j.micpath.2018.10.011. Epub 2018 Oct 10. PMID: 30316006.

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 *Trichoderma atroviride* AT10. Confidential information has been omitted.

Data Requirement	OCSPP (OPPTS) Guideline No.	Results Summary and Classification (As Applicable)	MRID No.
Product Analysis Data for the EP TRICOTEN WP (EPA File Symbol 96029-E…)			
Manufacturing Process	885.1200	Submitted data fulfill the requirement for manufacturing process. **Classification: Acceptable**	52159501
Deposition of a Sample in a Nationally Recognized Culture Collection	885.1250	Submitted data fulfill this requirement. **Classification: Acceptable**	51119701
Discussion of Formation of Unintentional Ingredients	885.1300	Submitted data fulfill this requirement. **Classification: Acceptable**	51119701
Analysis of Samples	885.1400	Submitted data fulfill this requirement. **Classification: Acceptable**	51119702
Color	830.6302	Pebble Gray **Classification: Acceptable**	51119705
Physical State	830.6303	Fine Powder **Classification: Acceptable**	51119705
Odor	830.6304	Characteristic **Classification: Acceptable**	51119705
Stability to Normal and Elevated Temperatures, Metals, and Metal Ions	830.6313	Data on elevated temperatures are not required since the product label contains language to store at 4^0 C. Data on stability to metals are not required since the product will not be stored in metal containers. **Classification: Acceptable**	51119705
Storage Stability	830.6317	Applicant reported storage stability data after storage of the product for 12 months at 4^0 C. **Classification: Acceptable**	51119705
pH	830.7000	4.29 **Classification: Acceptable**	51119705