OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

WASHINGTON, D.C. 20460

June 13, 2024

MEMORANDUM

SUBJECT: Product Chemistry Review and Human Health Assessment for a FIFRA Section 3 Registration of the Manufacturing-Use Product, (0178) Thyme Oil, Red, Containing 100% Red Thyme Oil as its Active Ingredient.

Action Code Case Number:	00132908
Submission Number:	1053153
EPA File Symbol Number:	94218-R (MP)
Chemical Class:	Biochemical
PC Code:	597801
CAS Number:	85085-75-2
Active Ingredient Tolerance/Exemption:	40 CFR180.950
MRID Numbers:	50898801-50898803; 50898811; 50898818-50898822; 51739501- 51739507; 51748601-51748602
PRIA Code:	B590

FROM: Risk Assessment Branch
Biopesticides & Pollution Prevention Division (7511M)

THRU:
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TO: Chris Pfeifer, Risk Manager
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*****This memorandum contains Confidential Business Information (CBI) which has been removed to the Confidential Appendix*****

ACTION REQUESTED

Biofungitek, S.L., requests registration of the manufacturing-use product (MP), (0178) Thyme Oil, Red (EPA File Symbol: 94218-R) containing 100% red thyme oil (*Thymus zygis*) as its active ingredient (AI). This species of thyme oil is also known as red thyme oil. In support of the registration for this new active ingredient, the applicant has submitted a proposed product label, a Confidential Statement of Formula (CSF) dated 06/03/2020, a data matrix dated 11/24/2021, and product chemistry data (MRID

50898801-50898803; 50898811; 50898821-50898822) and human health data and rationales (MRID 51739502-51739507; 50898818-50898820; 51748601-51748602).

EXECUTIVE SUMMARY

(0178) Thyme Oil, Red is a new MP containing 100% of the new active ingredient, thyme oil derived from *Thymus zygis* (hereafter, red thyme oil). In parallel with this submission, the registrant is seeking to register a second MP, Potassium Carbonate (99.5% Fine Powder) (EPA File Symbol: 94218-G), containing the new AI, potassium carbonate, and a new end-use product (EP), NSTKI-014 (EPA File Symbol: 94218-E). The proposed EP contains both new AIs and is a contact foliar fungicide used for application in residential/home gardens, turf, golf courses, greenhouses, orchards, nurseries, and fields for berries, bulb vegetables, citrus fruits, cole crops, corn, edible gourd, squashes, fruiting vegetables, leafy vegetables, pome fruits, and tree nuts among other crops in these groups.

Based on the exposure to red thyme oil from use as a pesticide, potential risk to humans is anticipated to be negligible. Sufficient toxicity data and rationales have been submitted by the applicant for registration of the proposed products. No endpoints have been identified based on the available toxicological data; therefore, the Agency conducted a qualitative risk assessment for dietary, residential, and occupational exposures. Red thyme oil is an edible oil; therefore, it is exempt from the requirement of a tolerance exemption under the EPA regulation described at 40 CFR 180.950(c). Because red thyme oil occurs naturally in the environment and humans are regularly exposed to it in food and cosmetic products, it is a mixture of organic compounds known to degrade rapidly via normal biological, physical, and chemical processes in the environment, it is already exempt from the requirement of a tolerance if used as an inert or active ingredient in pesticide chemical formulations, and there are no toxicological endpoints, the Agency does not anticipate any risks of concern for the proposed pesticidal use. Acceptable product chemistry data have been provided to support the applicant's request. All product chemistry and human health data requirements have been satisfied. Non-target organism data have been reviewed in a separate assessment.

The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/system/files/documents/2023-2/scientific_integrity_policy_2012_accessible.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.

1.0 Introduction

1.1 Biopesticide Use Pattern

Red thyme oil is proposed for use in both manufacturing- and end-use fungicide products. The MP cannot be used for direct treatment of pests and must be used only in EPs. The MP will be used to formulate fungicide EPs for use on specific agricultural crops, turf, and ornamentals. The proposed EP,

NSTKI-014 (EPA File Symbol: 94218-E), can be used for foliar application in residential/home gardens, turf, golf courses, greenhouses, orchards, nurseries, and fields for berries, bulb vegetables, citrus fruits, cole crops, corn, edible gourd, squashes, fruiting vegetables, leafy vegetables, pome fruits, and tree nuts among other crops in these groups. The EP is a wettable powder that is diluted in water and can be applied every 5-10 days at the first sight of disease symptoms but reduced to every 5 days after establishment of the disease. The EP is mixed at a rate of 2-7 pounds (lbs.) product in 100 gallons (gal.) of water to obtain a solution with a pH of 7-10. Since the AI is present at a nominal concentration of 1.75%, it is applied at a maximum application rate of 0.1225 lbs. AI/acre (0.001225 lbs. AI/gallon) for a single application; therefore, with only 10 applications allowed per year the total application rate will be 1.225 lbs. AI/acre/year. Applying the EP via any type of irrigation system is prohibited. The restricted-entry interval (REI) listed on the proposed label is 4 hours from the time of application. For the proposed EP, the registrant has included two sub-labels in addition to the main agricultural use label: one for residential turf/garden and another for turf and ornamental use by commercial applicators.

According to the sub-label for residential turf/garden uses, the EP is applied by mixing 2-7 teaspoons of product in a gallon of water per 450 square feet (ft^2). A minimum of 20 gallons of mixed spray solution of product can be applied per acre. The AI is applied at a rate of 0.126 lbs. AI/acre and 0.0013 lbs. AI/gallon solution per 450 ft^2. There are no baseline attire or personal protective equipment (PPE) requirements listed on the sub-label for the residential uses of the EP.

The second sub-label is for commercial use on residential lawns, golf courses and commercial turf grass sites, and the EP is mixed at a rate of 0.4-0.7 oz. of product in 1 gallon of water. Red thyme oil is applied via spray at a rate of 0.030 lbs. AI/acre and 0.0007 lbs. AI/gallon solution per 1000 ft^2. There are PPE requirements listed for these use sites on the second sub-label which match the agricultural use label. However, there is no REI listed for this sub-label.

All occupational applicators and handlers are required to wear PPE such as: long-sleeved shirts, long pants, shoes and socks, chemical-resistant gloves, and protective eyewear, which are listed on the proposed label and sublabel for commercial uses.

1.2 Anticipated Exposure Pathways

Based on uses proposed for the EP, the Biopesticides and Pollution Prevention Division (BPPD) anticipates short- and intermediate-term dermal and inhalation exposures to red thyme oil for occupational and residential handlers. There is potential for post-application exposure for agricultural workers re-entering treated areas. In addition, residential post-application exposures for adults (dermal) and children (incidental oral and dermal) are expected; however, residential, and non-agricultural label instructions recommend that users and applicators should wait until the spray is dry before re-entering the treated area. Non-occupational exposure resulting from spray drift from agricultural applications onto residential areas may also occur. Since red thyme oil is to be applied on food crops, there is the potential for dietary and drinking water exposures. Long-term (6 months to 1 year) non-dietary exposure is not expected for the proposed uses.

1.3 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations" (https://www.epa.gov/laws-regulations/summary-executive-order-12898-federal-actions-address-environmental-justice). As a part of every pesticide risk assessment, the Office of Pesticide Programs (OPP) considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, BPPD estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the U.S. Department of Agriculture under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas post-application are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

2.0 Product Chemistry

A. Active Ingredient Identity and Characterization

The biochemical pesticide red thyme oil, also known as Spanish thyme oil, is an essential oil extracted from the thyme plant species, *Thymus zygis.* It is a pale-yellow liquid that is classified as an edible oil, and it is used as a flavor and fragrance ingredient. Red thyme oil CAS No. 85085-75-2) is obtained from thyme plants grown in southern Spain and northern Africa. The primary components found in this active ingredient are thymol (~49-50%), γ-terpinene, p-cymene, linalool, and carvacrol. It is reported that some thyme oil from either *Thymus zygis or Thymus vulgaris* may contain up to 90% thymol (U.S. EPA, 2006).

The thyme oil species, *Thymus vulgaris,* is like the red thyme oil *Thymus zygis* species because both belong to the same botanical genus *Thymus* and same botanical family Lamiaceae and contain the same major constituent thymol (as a general comparison there is ~51 % for *Thymus zygis* oil and ~39% for *Thymus vulgaris* oil) (ECHA, 2020). The new TGAI, red thyme oil, is similar in chemical composition to the *Thymus vulgaris* species but differs by the amount of thymol present. The major constituent thymol is naturally found in food such as lime blossom honey and cooking herbs (e.g., oregano, carom, and basil) and foods derived from cranberry, mandarin, and tangerine oils. Based on these similarities, data for thyme oil (*Thymus vulgaris)* and thymol have been used in this assessment.

The essential oils of thyme from the plant species *Thymus zygis, Thymus vulgaris,* and *Thymus hyemalis* have strong antimicrobial properties, which make them useful in cosmetics, pharmaceuticals, and the food industry. Thyme oils are included in the flower oils class of biochemicals, which are generally applied at low levels in pesticide products, degrade rapidly in the environment, have a non-toxic mode

of action, and occur naturally. The FDA has categorized essential oils of thyme as Generally Recognized as Safe (GRAS) (21 CFR 582.20). As an edible oil, red thyme oil is exempt from the requirement of tolerance when used as an active ingredient in a pesticide chemical formulation if use is in accordance with good agricultural and manufacturing practices under 40 CFR 180.950(c).

B. Active ingredient/Manufacturing-Use Product

Sufficient data and information have been submitted to satisfy the product chemistry data requirements for the manufacturing-use product, (0178) Thyme Oil, Red. The TGAI is equivalent to the MP. The studies used to satisfy these requirements for the MP are summarized in the Data Evaluation Record (DER) which is included as a separate attachment. The product chemistry characteristics are summarized in Tables 1 and 2 below. Refer to the DER and Confidential Appendix for additional information.

TABLE 1. Product Chemistry Data for Thyme Oil, Red (40 CFR § 158.2030)

OSCPP Guideline	Study	Results	MRID
880.1100 830.1550	Product Identity and Composition	ACCEPTABLE	
880.1200 830.1650	Description of Starting Materials and Formulation Process	ACCEPTABLE	
880.1400 830.1670	Discussion of Formation of Impurities	ACCEPTABLE	
830.1700	Preliminary Analysis	ACCEPTABLE/GUIDELINE	
830.1750	Certified Limits	ACCEPTABLE/GUIDELINE	
830.1800	Enforcement Analytical Method	ACCEPTABLE/GUIDELINE	

TABLE 2. Physical and Chemical Properties of Red Thyme Oil (40 CFR § 158.2030)			
OCSPP Guideline	**Property**	**Description of Result**	**MRID**
830.6302	Color	Pale yellow	
830.6303	Physical State	Liquid	
830.6304	Odor	Thyme oil-like	
830.6313	Stability to Normal and Elevated Temperatures, Metals and Metal Ions	The MP was stable in the presence of metals, metal ions, and at both normal and elevated temperatures	
830.6315	Flammability		
830.6317	Storage Stability		
830.6319	Miscibility	Not required because the MP is not an emulsifiable liquid.	
830.6320	Corrosion Characteristics		
830.7000	pH		
830.7050	UV/Visible Light Absorption		
830.7100	Viscosity		
830.7200	Melting Point/Range	Not required because the MP is a liquid.	
830.7220	Boiling Point/Range		
830.7300	Density		
830.7520	Particle Size, Fiber Length and Diameter Distribution		
830.7550 830.7560 830.7570	Partition Coefficient (n-Octanol/Water)		
830.7840	Water Solubility		
830.7950	Vapor Pressure		

3.0 Human Health Risk Assessment

3.1 Toxicology Studies Available

To assess risks to human health from use of biochemical pesticides, BPPD requires a range of tiered toxicological data. Tier I data requirements for an active ingredient are:

- acute toxicity (acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, primary eye irritation, primary dermal irritation and skin sensitization);
- subchronic toxicity (90-day oral, 90-day dermal and 90-day inhalation);
- genetic toxicity (bacterial reverse mutation test and *in vitro* mammalian cell assay); and
- prenatal developmental toxicity.

Tier II and III testing (e.g., carcinogenicity, reproduction, and fertility effects, etc.) are triggered only when there is indication, usually through the lower tier testing, that a biochemical pesticide has unusual characteristics such as subchronic toxicity or being suspected or known to be a carcinogen.

The toxicology database is complete for biopesticide risk assessment for the proposed uses of red thyme oil. Tier II and Tier III studies have not been triggered and are not required at this time. To satisfy the human health assessment data requirements for the TGAI, the applicant submitted the following: 1) guideline acute toxicity studies and rationale, 2) rationales for the 90-day oral toxicity, 90-day dermal toxicity, 90-day inhalation toxicity, prenatal developmental toxicity, *in vitro* mammalian cell assay, bacterial reverse mutation test, and *in vivo* cytogenetics data requirements, and 3) a 26-week inhalation toxicity non-guideline study. The rationales for the 90-day oral, 90-day dermal, and 90-day inhalation requirements were accepted by OPP's Hazard and Science Policy Council (HASPOC) on December 14, 2022 (U.S. EPA, 2022).

All human health assessment data requirements have been satisfied to support this registration. Toxicology data and information are summarized in Table 3.

A. Toxicological Profile

Acute Toxicity

Acute toxicity data (acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, primary eye irritation, primary dermal irritation, skin sensitization) are required for biochemical TGAIs, MPs and EPs. To satisfy these data requirements, the registrant submitted guideline studies on the TGAI/MP in MRIDs 50898805-50898810 and 51739502-51739507. Red thyme oil is of low acute oral toxicity (Toxicity Category III) and inhalation toxicity (Toxicity Category IV) and shows moderate dermal toxicity (Toxicity Category II). It is a severe eye irritant (Toxicity Category II), moderate dermal irritant (Toxicity Category IV), and is considered to be a dermal sensitizer. DERs have been prepared and attached as separate documents for each human health assessment data requirement.

Subchronic Toxicity

<u>90-day oral toxicity</u>

Rationale was submitted for this data requirement (MRID 50898819) and was accepted by the HASPOC (U.S. EPA, 2022). The rationale is based on a weight of the evidence (WOE) approach that considered all of the available hazard and exposure information and included the following considerations: (1) two

repeat dose studies were performed on rats that were fed thymol (the major component in red thyme oil) at doses of 67 and 667 mg/kg/day for 19 weeks and 15.39, 30.78, and 61.55 mg/kg/day for 28 days. No adverse effects were observed in either study; (2) low acute oral toxicity (Toxicity Category III); (3) red thyme oil is naturally occurring and has been part of the human diet in food products and used in a variety of cosmetic products; (4) red thyme oil is categorized as an edible oil, so it is exempt from the requirement of a tolerance when used as an inert or active ingredient in pesticide chemical formulations (40 CFR 180.950); (5) the TGAI is a mixture of organic compounds known to be rapidly degraded in the environment to elemental compounds by normal biological, physical and/or chemical processes; and (6) thymol is currently exempt from the requirement of a tolerance in the U.S. in or on food commodities when applied/used in/on public eating places, dairy processing equipment, and/or food processing equipment and utensils and for residues in or on honey, honeycomb, and honeycomb with honey (40 CFR 180.1240). EPA waived the generic toxicological data requirements for thymol for these uses (U.S. EPA, 2010; 1993).

90-day dermal toxicity

Rationale was submitted for this data requirement (MRID 51748601) and was accepted by the HASPOC (U.S. EPA, 2022). The rationale is based on a WOE approach that considered all of the available hazard and exposure information, and included the following considerations: (1) thyme oil and thymol are naturally occurring with a history of use without adverse reactions seen from their uses in cosmetics and foods approved by the FDA; (2) the essential oils of thyme (including, *Thymus zygis*) are recognized as GRAS by the FDA (21 CFR 582.20); (3) there were no adverse effects observed in two thymol repeat oral (gavage) dose toxicity studies up to the highest dose tested (HDT) (667 mg/kg/day) or in the prenatal development toxicity study for thyme oil in rats dosed at 375 mg/kg/day (HDT); and (4) because the repeat-dose oral study approaches the limit dose with no reported adverse effects, endpoints were not selected, and a quantitative dermal assessment was not conducted.

90-day inhalation toxicity

Rationale was submitted for this data requirement (MRID 51748602) and was accepted by the HASPOC (U.S. EPA, 2022). The rationale is based on a WOE approach that considered all of the available hazard and exposure information, and included the following considerations: (1) low acute inhalation toxicity (Toxicity Category IV); (2) during a 26-week study, there were no adverse effects observed on body weights, organ weights, food intake, appearance, functional behaviors, or at the portal of entry in mice after repeated inhalation exposure to thymol concentrations of 0.1% and 0.5%, equivalent to dose levels of 0.171 mg/kg/week and 1.261 mg/kg/week respectively; (3) thyme oil's natural occurrence and its long history of human exposure in food and cosmetics; and (4) no adverse effects were observed close to the limit dose (1,000 mg/kg/day) based on the reported NOAEL of 667 mg/kg/day in the 19-week oral rat study; therefore, endpoints were not selected and a quantitative assessment was not conducted.

Chronic toxicity

Typically, chronic studies are not required for biochemical active ingredients. Currently, there are no chronic studies available for red thyme oil, and none are required at this time. There are no toxicological concerns anticipated.

Developmental and Reproductive Toxicity

There are no publicly available prenatal developmental studies for red thyme oil. In lieu of data on the TGAI/MP, a sufficient rationale (MRID 50898820) has been accepted based on the WOE approach that considered all of the available hazard and exposure information. This included the following considerations: (1) prenatal developmental studies were found in the public literature for the similar substances, thyme extract *(Thymus vulgaris)*, thyme oil, and thymol. In addition, the prenatal developmental toxicity of thymol has been previously reviewed by the HASPOC and rationale was accepted based its low acute oral toxicity, its extensive history of being part of the human diet, its existing tolerance exemption for use in/on food commodities, and no adverse effects reported in the repeat dose reproductive/developmental and safety assessment studies, (U.S. EPA, 2021); (2) all species of thyme oil (including red thyme oil) are naturally occuring and humans have a long history of exposure to these substances in food and cosmetic products; (3) low acute oral toxicity for red thyme oil (Toxicity Category III); (4) red thyme oil is categorized as an edible oil, so it is exempt from the requirement of a tolerance when used as an inert or active ingredient in pesticide chemical formulations (40 CFR 180.950); (5) in a non-guideline prenatal development study, essential oils were added to the diet of female mice at concentrations of 0.25% clove, 0.25% cinnamon, 0.25% thyme, 0.25% sage, and 0.1% oregano in a 1% edible soya oil over a two-week period. The body weights of the dosed female mice were evaluated on day 0, 7, and 14 and compared to the control group which were only fed the vehicle (1% edible soya oil) during the two-week period. The number of nuclei and distribution of nuclei were examined as the parameters to characterize the growth and development of preimplantation embryos; in addition, the percentage of normal and dead cells were examined to determine viability. There were no adverse effects on embryo development or on the 15 pregnant female mice that consumed the 0.25% thyme oil for two weeks at 375 mg/kg/day (Domaracky, 2007); and (6) a developmental safety assessment was conducted on pregnant rats who were administered thyme extract (*Thymus vulgaris)* and pure thymol. The thyme extract has the same major components, thymol and carvacrol, as found in the new active ingredient red thyme oil. In an Organization for Economic Cooperation and Development (OECD/OCDE) guideline study, three groups of rats were dosed daily via oral gavage at levels of 93.75, 937.5, and 1,875 mg/kg/day of thyme extract and at levels of 0.6, 6, and 12 mg/kg/day thymol in parallel, to evaluate rat fetus development from the 5th to 19th day of gestation. The study author noted there were no noticeable changes to maternal well-being or dam weights during the two-week dosing period. Postmortem examination of the rats dosed at 93.75, 937.5, and 1,875 mg/kg/day thyme extract, showed weight increases in the fetuses (~45%) of rats fed 1,875 mg/kg/day and in the placentas (29% -50%) at all three dose levels when compared to the control group. Further evaluation showed rats dosed with thyme extract at the highest dose tested (1,875 mg/kg/day) yielded a decrease in the number of implanted fetuses, an increased resorption index, and a lower number of live neonates. The study author concluded the increased weights were due to thyme extract being an appetite stimulant, which has been reported in previous studies (Dauqan, 2017). Nevertheless, there are uncertainties about this explanation, as food consumption information was not provided in this study, and the study authors reported that there were no changes in dam weights when compared to controls. The animal groups that were fed pure thymol did not exhibit the mean change in placental weights or fetal weights seen in the thyme extract groups. However, the thymol group fed at 0.6 mg/kg/day showed low fetal weights. On the 19th day all fetuses were inspected for external malformations, and teratogenic signs in the head, cleft, palate, limbs, or tail, but no adverse effects were found. Treatment at the three dose levels during in-life observations

of dams showed no external toxicity signs or mortality in all groups treated with thyme extract and thymol. The postmortem examination of the uteri of dams showed no abnormalities, and no significant differences in weights of uteri in all treated groups when compared to the control group. Only the animals dosed at 1,875 mg/kg/day (HDT), which is well above the recommended limit dose, showed adverse effects; at this high dosage level the lowest number of implanted fetuses were found. According to these results, the developmental NOAEL is 937.5 mg/kg/day and developmental LOAEL is 1,875 mg/kg/day due to the 50% decrease in mean live neonate number and higher percentage of early resorptions. For pure thymol the developmental NOAEL is 6 mg/kg/day and developmental LOAEL is 12 mg/kg/day based on the increase in the number of adsorbing sites in the uterus and reduced fetal body weights. According to the study authors, clinicians prescribe pregnant women thyme products at a dose equivalent to 93.75 mg/kg/day (based on 15g/100ml syrups); therefore, 93.75 mg/kg/day was considered the recommended thyme extract dose in this investigation (Tafesh, 2021).

Genotoxicity and Carcinogenicity

Genotoxicity data (MRID 50898818) are available on red thyme oil (*Thymus zygis*), and the two similar substances, thyme oil (*Thymus vulgaris*) and thymol. Publicly available data indicated that none of them were genotoxic. In an Ames test, all three substances tested negative and did not induce mutations in the *S. typhimurium* strains tested (ECHA, 2020; De Martino 2009; and LLana 2014). For the *in vitro* mammalian cell assays on mice and hamsters, both thyme oil (*Thymus vulgaris*) and thymol tested negative for mutations (Maisanaba, 2015; Hikiba, 2005). In addition, thyme oil (*Thymus vulgaris*) did not produce an increase in micronuclei in the bone marrow of rats during an *in vivo* micronucleus test. There are no carcinogenicity studies available for red thyme oil, as these studies are not typically required for biochemical pesticides and have not been triggered at this time.

TABLE 3. Human Health Assessment Data for Red Thyme Oil and Thymol (40 CFR § 158.2050).

OCSPP Guideline No.	Results	Toxicity Category/ Description	MRID or Source#
Acute oral toxicity (870.1100)	LD_{50} = 550 mg/kg (female rats) (HDT); Acceptable/Guideline	III	51739502
Acute dermal toxicity (870.1200)	LD_{50} = 2000-5000 mg/kg (male rats) = 200-2000 mg/kg (female rats) Combined = 2000 mg/kg Acceptable/Guideline	III (male rats) II (female rats) II (combined)	51739503
Acute inhalation toxicity (870.1300)	LC_{50} > 2.07 mg/L (male and female rats) Acceptable/Guideline	IV	51739504
Primary eye irritation (870.2400)	Severely irritating to the eye of rabbits. Corneal involvement cleared on Day 21. Acceptable/Guideline	II	51739505

TABLE 3. Human Health Assessment Data for Red Thyme Oil and Thymol (40 CFR § 158.2050).			
Primary dermal irritation (870.2500)	Moderately irritating, based on the primary irritation index (PII) of 3.8. Erythema and edema lessened from 24 to 72 hours post-administration. Hyperkeratosis, desquamation, and eschar were also noted with all effects clearing between 72 hours and 14 days after the test substance was administered. Based on the absence of moderate erythema at 72 hours, the test substance could fall in Toxicity Category IV; however, due to the PII and effects mentioned above, a Toxicity Category III classification is recommended for primary dermal irritation. Acceptable/Guideline	III	51739506
Dermal sensitization (870.2600)	The TGAI was a dermal sensitizer in mice (Local Lymph Node Assay) Acceptable/Guideline	Sensitizer	51739507
90-Day oral toxicity (870.3100)	<u>28-Day oral toxicity:</u> NOAEL > 61.55 mg/kg/day (HDT); **thymol** (rats) Dosed at 15.39, 30.78, and 61.55 mg/kg/day Acceptable/Non-Guideline <u>19-Week feeding study:</u> NOAEL > 667 mg/kg/day (HDT); **thymol** (rats) Acceptable/Non-Guideline		50898819 Gad, 2012 Hagan, 1967 US EPA, 2006
90-Day dermal toxicity (870.3250)	Rationale accepted by the HASPOC based on a WOE approach that includes a history of exposure to food and cosmetic products that contain thyme oil and thymol; the essential oils of thyme are granted GRAS status by FDA; and no endpoints have been selected because there were no adverse effects in the cited repeat oral dose studies. See Subchronic Toxicity section. Acceptable		51748601
90-Day inhalation toxicity (870.3465)	Rationale accepted by the HASPOC based on a WOE approach that includes a history of exposure to food and cosmetic products that contain thyme oil and thymol; the essential oils of thyme are granted GRAS status by FDA; no reported adverse effects in cited repeat inhalation and oral dose studies; and low acute inhalation toxicity. See Subchronic Toxicity section. <u>26-Week inhalation toxicity:</u> NOAEL > 1.26 mg/kg (repeat dose in mice 3x per week); **thymol** The mice were dosed at 0.1% and 0.5% which are equivalent to 0.171 mg/kg/week and 1.261 mg/kg/week respectively. Acceptable/Non-Guideline		51748602 Xie, 2019

TABLE 3. Human Health Assessment Data for Red Thyme Oil and Thymol (40 CFR § 158.2050).		
Prenatal Developmental toxicity (870.3700)	Maternal effects and embryonic development: **Thyme oil (*Thymus vulgaris)*:** Female mice dosed up to 375 mg/kg/day for two weeks showed no adverse effects. No detectable effects on embryo development were observed. Maternal NOAEL = 375 mg/kg/day (HDT) Developmental/Fetal NOAEL = 375 mg/kg/day (HDT) Acceptable/Non-Guideline **Thyme extract (*Thymus vulgaris)* and pure thymol:** Thyme extract: rats dosed at 93.75, 937.5, and 1,875 mg/kg/day for two weeks. Thymol: rats dosed at 0.6. 6, and 12 mg/kg/day for two weeks. Dams administered thyme extract and thymol showed no external signs of toxicity and no mortality during in-life observations. <u>Thyme extract (*Thymus vulgaris)*</u>: Developmental/Fetal NOAEL = 937.5 mg/kg/day Developmental/Fetal LOAEL = 1,875 mg/kg/day There was a decrease in fetal weights, increase in the resorption index, and a 50% reduction in mean live neonate numbers at 1,875 mg/kg/day <u>Thymol:</u> Developmental/Fetal NOAEL = 6 mg/kg/day Developmental/Fetal LOAEL = 12 mg/kg/day Acceptable/Guideline	50898820 Domaracky, 2007 Tafesh, 2021
Mutagenicity-Bacterial reverse mutation test (870.5100)	**Thyme oil (*Thymus zygis*):** Negative for reverse gene mutations in *Salmonella typhimurium* TA 1535, TA 1537, TA98, and TA100 in presence or absence of a metabolic system. Acceptable/Guideline **Thyme oil (*Thymus vulgaris*) and thymol:** In Ames tests, these compounds tested negative and did not induce mutations in the *S. typhimurium* strains tested. Acceptable/Guideline	ECHA, 2020 MRID 50898818 De Martino, 2009 LLAna-Ruiz-Cabello, 2014
Genotoxicity-*in vitro* mammalian cell assay (870.5375. 870.5900))	**Thyme oil (*Thymus vulgaris*) and thymol:** Negative for genotoxicity in the mouse lymphoma assay. Acceptable/Non-Guideline	US EPA, 2006 MRID 50898818
Genotoxicity-*in vivo* mammalian micronucleus assay (870.5395)	**Thyme oil (*Thymus vulgaris*):** Negative in mice and rats. Acceptable/Non-Guideline	Abdel-Aziem, 2014 MRID 50898818 US EPA, 2006
LD_{50} = median lethal dose; LC_{50} = median lethal concentration; HDT = highest dose tested; LOAEL = lowest-observed-adverse-effect-level; NOAEL = no-observed-adverse-effect-level #All data identified in the public literature were evaluated following the Agency's *Guidance for Open Literature Toxicity Studies to Support Human Health Risk Assessment.*		

B. Absorption, Distribution, Metabolism, and Elimination (ADME)

The absorption, distribution, metabolism, and elimination data collectively characterize the fate of a chemical once absorbed. These data are typically not required for biopesticide risk assessment and have not been triggered for the proposed use of red thyme oil.

C. Dermal Absorption

A dermal-absorption factor (DAF) is derived from dermal toxicity studies to determine the amount of dermal penetration that may occur if a compound were to get onto the skin. This factor is then applied within occupational and residential exposure scenarios. A DAF has not been determined for red thyme oil and is not needed at this time because a qualitative risk assessment has been conducted for the proposed use pattern of the active ingredient.

3.2 Safety Factor for Infants and Children (Food Quality Protection Act (FQPA) Safety Factor)

An FQPA safety factor is not required at this time for red thyme oil because EPA is performing a qualitative dietary assessment based on negligible toxicological and exposure concerns.

3.3 Toxicity Endpoint and Point of Departure Selections

No endpoints have been identified for red thyme oil.

3.4 Cancer Classification and Risk Assessment Recommendation

There are no carcinogenicity data available for red thyme oil and none are required at this time. The chemical is not considered to be genotoxic, based on the available *in vitro* and *in vivo* data.

4.0 Dietary Exposure (Food and Drinking Water) and Risk Assessment

Dietary exposure assessment is supported by residue chemistry data that are used to help estimate the exposure of the general population to pesticide residue in food and/or in feed. Residue chemistry data are also used to set and enforce pesticide tolerances. The typical data required for biochemical pesticides are:

- nature of the residue/metabolism (i.e., identification of the residue(s));
- residue analytical method (i.e., methodology for quantification of the residue(s));
- storage stability (i.e., stability of the residue(s) in a commodity when stored for an extended duration (>1 month) for later analysis); and
- magnitude of the residue (i.e., quantification of the residue(s) in a commodity).

Based on the available data and information, these data requirements have not been triggered for red thyme oil. Because the Agency has conducted a qualitative dietary risk assessment, residue data are not required.

Dietary risk assessment incorporates both exposure (food and drinking water) and toxicity of a given pesticide. In the case of red thyme oil, the Agency has conducted a qualitative dietary risk assessment

in lieu of a quantitative assessment. Dietary risk is expected to be negligible, as significant residues of the substance are not anticipated on treated commodities at the time of consumption based on its physical and chemical properties (i.e., TGAI is mixture of organic compounds that rapidly degrade in the environment to elemental compounds by normal biological, physical and/or chemical processes). Additionally, the AI is an edible oil that occurs naturally in food commodities and is of low toxicity. As an edible oil, red thyme oil is exempt from the requirement of tolerance when used as an active ingredient in a pesticide chemical formulation if its use is in accordance with good agricultural and manufacturing practices under 40 CFR 180.950(c). In addition, its major component, thymol, is currently exempt from the requirement of a tolerance in or on food commodities when applied/used in/on public eating places, dairy processing equipment, and/or food processing equipment and utensils (40 CFR 180.1240). Residues of thymol in or on honey, honeycomb, and honeycomb with honey are also exempt from the requirement of a tolerance when thymol is applied/used as a treatment to decrease the incidence of Varroa mite infestation in the honeybee (40 CFR 180.1240).

5.0 Residential (Non-Occupational) Exposure and Risk Characterization

The new MP is associated with a new EP, NSTKI-014. All EPs must be used in accordance with the directions on the label. Although there are proposed residential (non-occupational) uses, residential risk is anticipated to be minimal based on the following:

- Dilution of the EP in water will further reduce the concentration of the TGAI and minimize any potential exposure;
- Humans have a history of exposure to the TGAI because it is found in a variety of food and cosmetic products; it is an edible oil that has an established tolerance exemption; it is recognized as GRAS by the FDA; and there was a lack of systemic toxicity reported in the toxicological database.

Given these factors, when used according to the label directions, residential handler and post-application risk are not expected.

6.0 Non-Occupational Spray Drift Exposure and Risk Estimates

Spray drift is a potential source of exposure to those nearby pesticide applications. This is particularly the case with aerial application, but not applicable to the proposed EP based on the label. However, to a lesser extent, spray drift can be a potential source of exposure from the ground application methods (e.g., groundboom) employed for the proposed EP, NSTKI-014. To further minimize risk and exposure to spray drift, according to the label the use of shielded sprayers is highly recommended. A qualitative risk assessment has been conducted for the proposed use of red thyme oil in NSTKI-014 and is based on the physical chemical properties of the active ingredient and history of human exposure. The TGAI red thyme oil and its major constituent, thymol, are naturally occurring with a history of use without significant adverse reactions seen from their uses in cosmetics and foods approved by the FDA. In addition, these essential oils have been granted GRAS status by the FDA (21 CFR 582.20). Further, in the toxicology database, there were no adverse effects in repeat oral (gavage) dose toxicity studies up to 667 mg/kg/day (HDT) for thymol, and none reported in prenatal development toxicity studies for thyme extract up to 937.5 mg/kg/day. Since the repeat-dose oral toxicity studies approached the limit dose with no adverse effects identified, endpoints were not selected.

7.0 Aggregate Exposure and Risk Characterization

In accordance with the FQPA, OPP must consider and aggregate (add) pesticide exposures and risks from three major sources: food, drinking water, and residential exposures. In an aggregate assessment, exposures from relevant sources that have the same toxicological endpoints are added together and compared to quantitative estimates of hazard, or the risks themselves can be aggregated. When aggregating exposures and risks from various sources, OPP considers both the route and duration of exposure.

Based on the available data and information, the Agency has concluded that a qualitative aggregate risk assessment is appropriate to support the pesticidal use of red thyme oil, and that risks of concern are not anticipated from aggregate exposure to the substance. This conclusion is based on the low toxicity of the active ingredient, its classification as an edible oil, its use in food and cosmetic products, and the existing natural levels found in thyme plants throughout the environment.

8.0 Cumulative Exposure and Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to red thyme oil and any other substances, and this biopesticide do not appear to produce a toxic metabolite produced by other substances. For the purposes of this action, EPA has not assumed that this active ingredient has a common mechanism of toxicity with other substances. In 2016, EPA's Office of Pesticide Programs released a guidance document entitled, *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis* [https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework]. This document provides guidance on how to screen groups of pesticides for cumulative evaluation using a two-step approach beginning with the evaluation of available toxicological information and if necessary, followed by a risk-based screening approach. This framework supplements the existing guidance documents for establishing common mechanism groups (CMGs) and conducting cumulative risk assessments (CRA). During Registration Review, the Agency will utilize this framework to determine if the available toxicological data for powdered corn cobs suggests a candidate CMG may be established with other pesticides. If a CMG is established, a screening-level toxicology and exposure analysis may be conducted to provide an initial screen for multiple pesticide exposure.

9.0 Occupational Exposure and Risk Characterization

Short- (1 to 30 days) and intermediate-term (1 to 6 months) dermal and inhalation exposures to red thyme oil are expected for occupational handlers from application of the proposed product. There is potential for post-application exposure for agricultural workers re-entering treated areas. Occupational handlers are required to wear the following PPE: long-sleeved shirts, long pants, shoes, and socks, chemical resistant gloves, and protective eyewear which are listed on the proposed label. The types of application equipment include groundboom with the option of adding a shield over the sprayers. Although they are not required according to the EP label, shielded sprayers are highly recommended because it will help reduce drift and exposure. There is a 4-hour REI for occupational workers. Occupational handler risk and post-application risk to red thyme oil are expected to be negligible based on the natural occurrence and long history of human exposure, low application rates, physical chemical

properties (the TGAI is a mixture of organic compounds known to be rapidly degraded in the environment to elemental compounds by normal biological, physical and/or chemical processes), lack of toxicological concerns, and PPE requirements; therefore, a qualitative risk assessment has been conducted in lieu of a quantitative assessment.

10.0 Tolerance Exemption

A new petition is not required as the AI is exempt from the requirement of a tolerance under 40 CFR 180.950(c), as an edible oil. According to the food-grade certification and proposed label, the MP meets the criteria of the exemption.

RECOMMENDATIONS AND CONCLUSIONS

1. The submitted product chemistry data are **ACCEPTABLE**. All data requirements have been satisfied and no additional information is required.

2. The submitted basic CSF and data matrix, dated 06/03/2020 and 11/24/2021, respectively, for MP (0178) Thyme Oil, Red are both deemed **ACCEPTABLE.**

3. Human health assessment data and information submitted by the applicant for the proposed TGAI/MP are **ACCEPTABLE**. No additional data are required, and no risks of concern have been identified for the proposed uses of red thyme oil.

Note to Risk Manager:

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MRID 50898802: ACCEPTABLE
MRID 50898803: ACCEPTABLE
MRID 50898811: ACCEPTABLE
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MRID 51748601: ACCEPTABLE
MRID 51748602: ACCEPTABLE
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MRID 51739503: ACCEPTABLE
MRID 51739504: ACCEPTABLE
MRID 51739505: ACCEPTABLE
MRID 51739506: ACCEPTABLE
MRID 51739507: ACCEPTABLE
MRID 50898805: *Superseded by 51739502*
MRID 50898806: *Superseded by 51739503*
MRID 50898807: *Superseded by 51739504*
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