United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Natamycin
Crops

Identification of Petitioned Substance

Chemical Names:
$C_{33}H_{47}O_{13}N$

16-(3-Amino-3,6-didesoxy-beta-D-mannopyranosyloxy)-5,6-epoxy-8,12,14-trihydroxy-26-methyl-2,10-dioxo-1-oxacyclohexacosa-3,17,19,21,23-pentaen-13-carbonsaeure

22-((3-amino-3,6-dideoxy-beta-D-mannopyranosyl)oxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo(22.3.1.0(sup 5,7))octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

(1R,3S,5R,7R,8E,12R,14E,16E,18E,20E,22R,24S,25R,26S)-22-[(3-amino-3,6-dideoxy-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.05,7]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

Other Name:
Natamicina; Natamycine; Natamycinum; Pimaricin; Pimaricine; Pimarizin; Tennecetin

Trade Names:
BioSpectra 100SC; BioShield 100SC; Natamycin L; Nature's Shield 100SC; Zivion M; Zivion P; Zivion S

CAS Numbers:
7681-93-8

Other Codes:
Antibiotic A-5283
EINECS 231-683-5
FDA UNII: 8O0C852CPO
E 235
INS 235
CL 12,625

Summary of Petitioned Use

Natamycin is used as a fungicide in mushroom production and as a post-harvest handling treatment of raw agricultural commodities to control fungal diseases. In 2016, a petition for classification of natamycin as an allowed nonsynthetic substance in organic production was submitted for review by the National Organic Standards Board (NOSB) (Technology Sciences Group, Inc. 2016). This technical report supports the NOSB's review of this petition and addresses specific focus areas requested by the NOSB Crops Subcommittee:

- Materials used in manufacture of natamycin that may include: soy protein isolate, ammonium sulfate, sodium nitrate, or beef extract (as nitrogen sources in the substrate); defoamers; pH adjuster (potassium hydroxide); yeast; bulking agents (xanthan gum); salt**.** *(See Evaluation Question #1)*
- Natamycin is usually applied with water or with a wax or oil in post-harvest handling. Provide information on how long it may remain on the food, or how quickly it breaks down (in darkness, UV or fluorescent light) *(See Evaluation Question #4)*
- Natamycin is "exempt" from any specific limitation on amount used in post-harvest handling, but has a 6 hour application to harvest time for mushrooms; need further information on why exempt and why a withdrawal time for mushrooms? Also, there is a limit to the amount used in cheese and meat products (acceptable Daily Intake allowed in cheese or processed meats (.3mg/kg) 20 ppm in the finished product). *(See Approved Legal Uses of the Substance)*
- Purity of natamycin is 98.17% or 98.27%, what is the remainder? What are the "other ingredients" in the two brand name products named in the petition, as well as any other brand name products containing natamycin for these petitioned uses? *(See Combinations of the Substance)*
- Does long term use lead to fungal resistance to natamycin? Are there horizontal gene transfer resistance issues with similar substances to natamycin? How widespread is its current use in

nonorganic mushroom production or post-harvest handling? How long has it been in use on nonorganic mushrooms and post-harvest handling? Fungal resistance and human health effects have been reviewed based on the use only on cheese and meat products, so knowing how long and how widespread the use is in mushrooms and post-harvest handling would be informative. *(See Historic Use and Evaluation Question #8)*

- Natamycin is used in human health to control fungal infections in the eye, and related very closely to an antibiotic used for vaginal candida. Need to also research effect on human intestinal flora. Also used in livestock to control ringworm. Are there other human or livestock health uses for natamycin, and any possible issues between this human health use and the petitioned use? *(See Evaluation Question #10)*

Note: Natamycin is referred to as both a fungicide and a fungistat in the literature. Under the strictest definition, a fungicide is a substance that kills fungi, whereas a fungistat is a substance that inhibits the growth of fungi (Mehrotra 2013). Under this definition, natamycin is a fungistat (*see Action of the Substance)*. The EPA more broadly defines a fungicide as a "chemical for the control of fungi" (EPA 2007a). Except when referred to specifically as such within literature, natamycin will be referred to under the broader definition (as a fungicide) within this report.

Characterization of Petitioned Substance

Composition of the Substance

Natamycin is composed of a macrocyclic lactone (large ring, Figure 1), and the amino-glycoside, mycosamine (small ring) (Brik 1976). Lactones are characterized by the presence of oxygen within the backbone of the ring, which originates from the reaction of a hydrocarbon chain with an alcohol (Bruice 2001). Furthermore, the lactone ring in natamycin contains a series of four alternating single and double bonds. The electrons from these bonds are distributed across the bond pairs equally, forming a region known as a "polyene,[1]" which is associated with unique physical and optical characteristics (Hamilton-Miller 1973). Molecules that follow this basic structural motif are termed polyene macrolides.

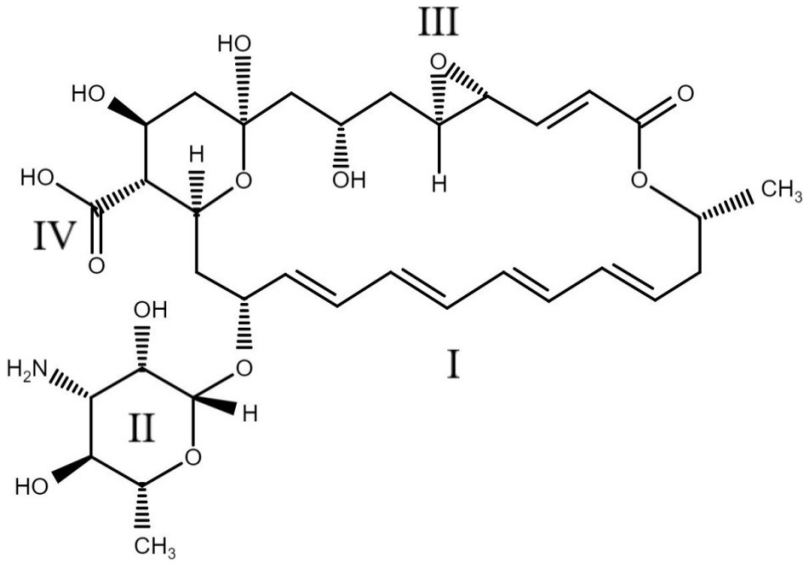

Figure 1: Chemical Structure of Natamycin, adapted from the National Library of Medicine (U.S. National Library of Medicine 2017a). Note the conjugated bonds forming the tetraene moiety, (I) which gives natamycin its optical properties; mycosamine, (II) which may contribute to natamycin antifungal activity; and the epoxide moiety (III) and carboxylic acid (IV) that are changed during acid degradation.

[1] Natamycin is more specifically a "tetraene" when one counts the specific number of bond pairs (four).

Source or Origin of the Substance

Natamycin is a naturally occurring compound produced by several soil bacteria including *Streptomyces natalensis* (Struyk, et al. 1957-1958), *S. chattanoogensis* (Martín and Aparicio 2009), *S. gilvosporeus* (Chen, Lu and Du 2008), and *S. lydicus* (Atta, et al. 2015). The European Food Safety Authority (EFSA) describes *Streptococcus lactis* producing natamycin (EFSA 2009); however, this source was not identified elsewhere in published literature. Commercial natamycin is produced from *S. natalensis*, and *S. gilvosporeus* primarily (VGP 2015). Natamycin is commercially produced using submerged aerobic fermentation with subsequent extraction and purification steps (*see Evaluation Questions #2 and #3).*

Properties of the Substance:

As a crystalline powder, natamycin is white to creamy in color (Brik 1994). The molecule has low solubility in water at a neutral pH, but dissolves at pH extremes (e.g., lower than pH 4.0, and above 10.0) (Brik 1981). It is soluble in organic solvents, such as alcohols, glycols, or formaldehyde (Struyk, et al. 1957-1958) (Burns 1959). Natamycin, like other polyene macrolides, is amphoteric (it can act as an acid or a base) but is neutral between pH 5.0 and 9.0 (Hamilton-Miller 1973). The carboxyl (Figure 1, IV) and the mycosamine groups (Figure 1, II) contribute to the amphoteric properties of the molecule (te Welscher, ten Napel, et al. 2008), with both becoming protonated at low pH, yielding a molecule with net positive charge (Koontz, et al. 2003). The low solubility of natamycin is considered advantageous in food surface applications because the substance will remain where it is applied, and not significantly migrate into the food (Stark and Tan 2003). For instance, after 28 days in Tilsiter cheese, natamycin migrated only 2.6mm (Kiermeier and Zierer 1975). The physical and chemical properties of natamycin are summarized in Table 1.

Table 1. Physicochemical Properties of Natamycin

Property	**Value**[a]
Physical state	Solid
Appearance	White to cream colored crystalline powder
Odor	None
Molecular weight	665.75 (g/mol)
Melting point	290°C
Water solubility	~30-100 ppm
pH	5-7.5
Density	303-588 g/L (loose vs. packed)

[a] **Sources: (Brik 1981), (Stark and Tan 2003), (Jones 2011)**

Natamycin can form three known crystal lattice structures: the commonly occurring alpha, and the less common and more heavily manipulated delta and gamma forms. These forms of natamycin are relatively stable in the absence of light. Alpha-natamycin crystals can be either hydrated, or dried further to form an anhydrous material. The commonly occurring trihydrate form (crystals containing three water molecules per natamycin) is more stable than the anhydrous form (Borden, Maher and Sklavounos 1999). Alpha-natamycin crystals are known to occur in two shapes: plates, and needles. Plate-shaped crystals are formed in standard manufacturing processes (described in responses to *Evaluation Question #2*). Needle-shaped crystals are formed by dissolving previously obtained natamycin crystals in water at either high or low pH (more than 10.0 or less than 4.0), followed by neutralization of the media over a period of 5-50 minutes and at temperatures between 5 and 35°C (De Haan and Van Rijn 2013).

Delta-natamycin is known to occur under specific manufacturing processes (van Rijn, et al. 1998). Delta-natamycin can be converted into another unique form, the trihydrate gamma-natamycin (not to be confused with the commonly occurring alpha-natamycin trihydrate, or simply natamycin). Delta-natamycin is anhydrous, and is more stable than anhydrous alpha-natamycin. Gamma-natamycin (a trihydrate) is also stable, and has enhanced bioactivity against some fungal species. Both delta and gamma crystals revert to alpha-natamycin after recrystallizing in water (van Rijn, et al. 1998).

Commercially available forms of natamycin are most likely in the (more stable) form of trihydrates (Stark and Tan 2003). Unless otherwise stated, the remainder of this report will address natamycin in the alpha crystalline trihydrate form.

Specific Uses of the Substance:

Natamycin is used for its antifungal properties, and is active over a wide pH range. Burns (1959) found that natamycin was active against *Saccharomyces carlsbergensis* from pH 4.0 to 10.0. It is effective against yeasts such as *Candida albicans, Cryptococcus neoformans* and *Saccaromyces cerevisiae*, and filamentous fungi such as *Aspergillus flavus, Penicillium chrysogenum, Trichoderma spp.*, and *Paecilomyces spp*. as well as many others (Struyk, et al. 1957-1958). Natamycin also demonstrates activity against parasitic protozoa, such as *Trypanosoma cruzi* (causal agent of Chagas disease) which, like many fungi, contain ergosterol in their cell membranes (Rolón, et al. 2006). While no longer considered within the fungi kingdom, oomycetes (such as the causal agent of Potato Late Blight, *Phytophthora infestans*) are notably insensitive to natamycin (Judelson and Blanco 2005) (WHO 2001).

Commercial applications of natamycin in crop, livestock, and food production can be grouped into three basic categories: 1) as an agricultural fungicide, either pre- or post-harvest, 2) as a livestock medication, and 3) as a preservative in processed foods.

Fungicide in agriculture

Natamycin is used to control fungal diseases in enclosed mushroom production facilities (EPA 2012a). EPA-approved labels include its use in the control of dry bubble disease, caused by *Lecanicilium fungicola* (also known as *Verticillium fungicola*), which affects commercially grown button mushrooms (*Agaricus bisporus*). The disease does not affect the vegetative portion of the fungus, but rather the edible mushroom, causing lesions and tissue disruption (such as stipe "blow-out" and other deformations). Natamycin may also be applied to mushrooms during production in an aqueous solution by hand or with an automatic watering system.

Natamycin is used as a post-harvest fungicide on fruit (including citrus, berries, pomes, stones, pineapples, melons, and bananas) to prevent spoilage caused by fungi such as *Penicillium spp.* and *Geotricum spp.* (Pace International 2016) (Huang, et al. 2016). Application methods vary depending on the label instructions and generally include first mixing with water or wax (see *Combinations of the Substance* for more information). Fruit application methods include dipping, drenching, spraying, and flooding (EPA 2017a).

Medical uses for livestock

Natamycin is used in animal health care applications as a veterinary drug. It has moderate activity against dermatophytes, yeasts and *Aspergillus*. It is used in some parts of the world to treat ringworm and candidosis in horses and cattle (Rochette, Engelen and Vanden Bossche 2003), and has also been used to treat nasal aspergillosis in horses. It is approved for use as an additive for feed and drinking water of broiler chickens (EPA 2012a).

Preservative in processed foods

Natamycin is commonly used in the U.S. to protect the surface of cheese and, in Europe and other countries, sausages against fungal development (Streekstra, Verkennis, et al. 2016). Natamycin is marketed for use in products such as cottage cheese, sour cream, yogurt, and packaged salad mixes (Siveele B.V. 2009). It is used in beverage products to prevent mold and yeast (Keefe 2015).

Approved Legal Uses of the Substance:

Approved uses in agriculture (pre and post-harvest)

Natamycin used as petitioned is regulated by the EPA. Antifungal products with natamycin as an active ingredient are subject to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and therefore must be registered with the EPA. Natamycin was approved by the EPA in 2012 for use as a fungistat on mushrooms grown in enclosed mushroom growing facilities (EPA 2012a). In 2016, the EPA further

approved its use in post-harvest facilities to control fungal disease on additional specified crops (EPA 2016b).

Natamycin is exempt at 40 CFR 180.1315 from the requirement of a tolerance for residues in or on mushrooms, pineapples, citrus, pome, stone fruit crop groups, avocado, kiwi, mango, and pomegranates when used in accordance with label directions and good agricultural practices. Natamycin's exemption from the requirement for a tolerance is based on the determination of EPA's Biopesticides and Pollution Prevention Division that data on the product chemistry and toxicity satisfy the current guideline requirements for tolerance exemption (EPA 2012a). For more information on toxicity, see *Evaluation Question #10*.

The EPA-approved label for Natamycin L includes use instructions for a 6-hour waiting period, or pre-harvest interval[2] (PHI), between application and harvest of mushrooms, whereas no PHI is indicated for other antifungal fruit wash uses because it is applied post-harvest. Originally, the label for Natamycin L approved by the EPA in 2012 included a 4-day (96-hour) PHI for mushrooms (EPA 2012c). In 2013, the EPA approved a shortening of the PHI to 6 hours as well as a shortening of the steaming required for spent mushroom media from 24 to 12 hours (EPA 2013). Information submitted to the EPA regarding the basis for the PHI or its shortening is not publicly available. In 2016, the label was amended to include post-harvest use on citrus, pome and stone fruit crops, avocado, kiwi, mango and pomegranate (EPA 2016b).

Approved uses in livestock production

Natamycin is listed in FDA regulations under 21 CFR 573.685 as an additive in broiler chicken feeds according to stated specifications, which detail use of the additive as part of a premix with calcium carbonate and lactose, used for retarding the growth of *Aspergillus parasiticus*. Levels for components in the premix are set and feed rates are specified to equal 11 ppm natamycin.

Natamycin is also approved by the FDA as an ophthalmic suspension under the New Drug Application number 050514 to suppress fungal eye infections such as blepharitis, conjunctivitis, and keratitis per FDA regulations at 21 CFR 449.40.

Approved uses in food processing

The FDA permits natamycin as a direct food additive at 21 CFR 172.155 for application on cheese as an antimycotic to inhibit the growth of yeast and mold. The listing includes specifications for purity (must be 95-99 percent pure, on an anhydrous basis) and limits heavy metal contaminants. It also limits natamycin content in finished cheese to 20 mg/kg.

Natamycin is also recognized by the FDA as Generally Recognized as Safe (GRAS) when used to prevent growth of food spoilage molds in yogurt at a minimum level not to exceed 5 mg/kg natamycin (FDA 2014), and also when used in ready-to-drink tea beverages, fruit flavored fruit-flavored energy drinks, sport and isotonic drinks, and fruit-flavored beverages at levels not to exceed 5 ppm (FDA 2015).

Action of the Substance:

Natamycin has two primary modes of action: inhibition of fungal growth and inhibition of mycotoxin production.

Inhibition of fungal growth

Natamycin's best known mode of action involves inhibition of fungal growth. Natamycin is effective against a wide array of fungi (Struyk, et al. 1957-1958), and disrupts normal cell membrane function by interfering with ergosterol (te Welscher, ten Napel, et al. 2008). Ergosterol is critical to fungi that contain it, as it is involved in a wide array of cellular processes, including growth (Parks and Casey 1995). When

[2] Pre-harvest interval is defined by the EPA as "the time between the last pesticide application and harvest of the treated crops" (EPA 2009).

ergosterol is blocked, fungal cells are unable to transport materials such as glucose and amino acids across cell membranes (te Welscher, van Leeuwen, et al. 2012).

Ergosterol is found in many (though not all) fungal cell membranes (Weete, Abril and Blackwell 2010) and the level of ergosterol in fungi fluctuates over time, across species, and at different developmental stages (Pasanen, et al. 1999). For example, during spore germination, the amount of ergosterol can increase more than four times in six hours (van Leeuwen, Smant, et al. 2008).

Much of the research on natamycin focuses on its effect on fungal spores, as opposed to mature vegetative tissue (hyphae). Natamycin's interference with the normal function of ergosterol inhibits the active uptake of vesicles (endocytosis, a fission process) (van Leeuwen, Golovina and Dijksterhuis 2009) and also affects the membrane fusion process of organelles (vacuoles), acting before cell membranes even contact each other (te Welscher, Jones, et al. 2010). Endocytosis and exocytosis are thought to be important elements in fungal germination and growth, and growth in fungi occurs in regions that are rich in sterols (such as ergosterol). Natamycin's interference with ergosterol is also associated with changes in the regulation of cell membrane proteins, such as sugar and amino acid transporters (te Welscher, van Leeuwen, et al. 2012). These changes block the uptake of nutrients by fungal spores, and in response, the fungi up-regulate the production of cell membrane proteins in order to attempt to overcome the nutrient shortage (te Welscher, van Leeuwen, et al. 2012). However, the researchers (te Welscher, van Leeuwen, et al. 2012)found that the effects of natamycin were reversible in *Aspergillus niger* and *Saccharomyces cerevisiae*, indicating that up-regulation of these proteins may not lead to lasting effects in these species.

Other polyene antimycotics such as amphotericin B, and nystatin (a tetrane), have been shown to form pores that increase the permeability (or "leakiness") of fungal cell membranes in addition to interfering with ergosterol (Aparicio, et al. 2016). This same mode of action was described in the 2006 Technical Report on Natamycin (ICF International 2006). Since 2006, understanding regarding natamycin's activity has progressed; unlike the other polyene antimycotics, it is now believed that natamycin does not form pore complexes that create leaks in cell membranes (te Welscher, ten Napel, et al. 2008).

The effect of natamycin on fungal membranes is substantial. The minimum inhibitory concentration (MIC), or the amount of natamycin needed to prevent growth against its targets is very low. For example, the MICs for isolates of *Penicillium, Mucor, Rhizopus, Paecilomyces, Fusarium*, and *Trichoderma* from commercial poultry feed ranged from 2.15 to 5.80 ppm (Brothers and Wyatt 2000). Some species, such as *Aspergillus spp.* tend to be more naturally tolerant of natamycin. The lower solubility estimate of natamycin in water at 30 ppm (Brik 1981), while low, exceeds the MIC for susceptible fungal targets. As levels of natamycin decrease due to diffusion, degradation, and absorption by fungi, natamycin is released from natamycin crystals into the surrounding substrate (Stark and Van Rijn 2010). This effectively balances the aforementioned losses and maintains concentrations that exceed the MIC for target species.

Inhibition of mycotoxin production

Fungi that contaminate food can produce mycotoxins. Minute levels of natamycin (1 ppm) can inhibit the production of aflatoxin B_1, ochratoxin, penicillic acid, and patulin (Ray and Bullerman 1982). Ray noted that natamycin's effect on mycotoxin inhibition is greater than its effect on fungal growth (see below). For example, a 10 ppm treatment of natamycin reduced growth of *Aspergillus ochraceous* by 46 percent, but reduced ochratoxin production by 100 percent. Research demonstrating the mechanism by which natamycin acts to reduce mycotoxin production was not found. It may be that the interference with membrane trafficking has a corresponding effect on mycotoxin production.

Assessment of whether natamycin acts as an antibiotic

The literature has established that natamycin is ineffective against bacteria (Struyk, et al. 1957-1958) (Burns 1959) (Brik 1981) (WHO 2002) due to the negligible presence of ergosterol in bacterial membranes (Aparicio, et al. 2016). With the exception of the EPA, most regulatory agencies would exclude natamycin from their respective definitions of "antibiotic" because natamycin has no effect on bacteria. Regulatory definitions from FDA and USDA would classify natamycin as an antimicrobial instead of an antibiotic.

The EPA's definition for antibiotics covers a broader variety of substances than most other regulatory agencies. The EPA defines antibiotics as: "*A metabolic product of one microorganism or a chemical that in low concentrations is detrimental to activities of specific other microorganisms. Examples include penicillin, tetracycline, and streptomycin. Not effective against viruses. A drug that kills microorganisms that cause mastitis or other infectious disease*" (EPA 2007b). The EPA's definition of the term "antibiotic" encompasses natamycin, as natamycin is a metabolic product of a microorganism (bacteria) that is detrimental to other microorganisms (fungi). When natamycin was specifically reviewed for use as a pesticide ingredient to control the germination of mold and yeast spores in mushroom substrates, the EPA stated that it was a fungistat, and a naturally occurring antimycotic compound. When describing its manufacture, they referred to it as an antibiotic (EPA 2012a).

While an explicit definition of "antibiotic" from the FDA could not be found, they state that "*Antibiotics are meant to be used against bacterial infections*" (FDA 2011). When natamycin is used as a drug, it is excluded from the FDA's implicit definition of an antibiotic as it has no activity against bacteria. Instead, it would fall under the term "antimicrobial": "*Antimicrobial drugs include all drugs that work against a variety of microorganisms, such as bacteria, viruses, fungi, and parasites. An antibiotic drug is effective against bacteria. All antibiotics are antimicrobials, but not all antimicrobials are antibiotics*" (FDA 2017).

Additionally, under the definition used by the USDA One Health Joint Working Group,[3] natamycin would be considered antimicrobial: "*…antimicrobial drugs are a broader category since they have activity against more than just bacteria and include synthetic medications such as sulfonamides*" (USDA 2014).

As with the FDA and USDA's use of the term, natamycin would be excluded from the definition of antibiotics by the World Health Organization (WHO) as it is not used to prevent or treat bacterial infection: "*Antibiotics are medicines used to prevent and treat bacterial infections*" (WHO 2016).

Combinations of the Substance:

With respect to the petitioned use, natamycin is not known to be a precursor to--or a component of--other synthetic substances on the National List at §205.601. Purified natamycin on its own is not currently sold for use as an agricultural fungicide, but is sold for further formulation. Commercially available natamycin products for agricultural use are formulated with other ingredients, as described below. Label instructions for some products require the applicator to first mix the natamycin product with water or wax. Further details on the type or identity of wax are not specified.

As of July 2017, there are eight EPA-registered natamycin products for use in enclosed mushroom production facilities or as a post-harvest fungicide. Since natamycin must be registered with the EPA, it is expected that these are the only commercially available products available for use in the U.S. for the petitioned uses. There are three EPA registration numbers associated with these eight products (see Table 2), each with natamycin as the reported active ingredient (EPA 2017a). All EPA registrations are held by DSM Food Specialties.

[3] The USDA One Health Joint Working Group includes the Animal and Plant Health Inspection Service (APHIS), Agricultural Research Service (ARS), Food Safety and Inspection Service (FSIS), Economic Research Service (ERS), National Agricultural Statistics Service (NASS), and the National Institute of Food and Agriculture (NIFA) (USDA 2014).

Table 2: Summary of EPA registered natamycin products as of July, 2017.

EPA Reg. No.	Number of registered products	Natamycin	Other ingredients	Product description
87485-1	1	91.02%	8.98%	Technical Grade of the Active Ingredient (TGAI) intended for formulating into fungicidal products
87485-2	6	10.34%	89.66%	For use on mushrooms; citrus; pome and stone fruit; avocado; kiwi; mango; pomegranate
87485-3	1	4%	96%	For use on pineapple

EPA Reg. No. 87485-1

This product has a purity of 91.02 percent natamycin. The composition of the other ingredients is not disclosed on the product label. In the petition, Technology Sciences Group, Inc. states that the product does not contain any ancillary substances, but that impurities may be present such as water of hydration, naturally occurring natamycin-related by-products co-extracted with the natamycin, residual solvent, and natamycin degradates (Technology Sciences Group, Inc. 2016). Therefore, the 8.98 percent other ingredients are expected to be composed of these substances, with the majority being composed of water of hydration, which makes up the natamycin trihydrate structure.

EPA Reg. Nos. 87485-2 and 87485-3

Natamycin is the only active ingredient in formulated products with EPA Reg. Nos. 87485-2 and 87845-3. Other ingredients used to formulate the products are not disclosed on labels or available Safety Data Sheets (SDS).

Formulation information for specific products within the scope of the petitioned use is not publicly available; however, formulants identified in natamycin patents are listed in Table 3. Many (but not all) of these substances are present on the 2004 EPA List 4, which indicates that they would be permitted as inerts under the NOP regulations in accordance with §205.601(m). They include pH adjustors and buffering agents (e.g., citric acid), thickening agents (e.g., xanthan gum), fillers (e.g., lactose), surfactants (e.g., sodium lignosulfonate), antifoaming agents (e.g. vegetable oils), and solvents (e.g., ethanol).

Table 3: Formulants noted in patents for agricultural uses of natamycin.

Patent holding company	U.S. Patent Number (and source)	Product form	Uses	Formulants
Gist-Brocades B.V.	5,552,151 (Noordam, et al. 1996)	Wettable powders for making suspensions	Non-specific agricultural products	*Thickening / bulking agents*: xanthan gum[iv], carrageenan[iv], methylcellulose[iv], gum Arabic[iv]. *Surfactants*: sodium dodecyl sulfate[iv] *Buffers:* citric acid[iv], mono[iv]-, di[iv]-, tri-sodium salts of citric acid[iv], mono[iv] and disodium salts of phosphoric acid[iv] *Fillers:* lactose[iv] or cellulose[iv]
Gist-Brocades B.V.	5,821,233 (van Rijn, et al. 1998)	Metallic salts and alternate crystal structures	Food preservation, agricultural products, pharmaceutical	*Carriers*: Fumed silica[iv], microcrystalline cellulose powder[iv].

Patent holding company	U.S. Patent Number (and source)	Product form	Uses	Formulants
DSM IP Assets, B.V.	7,816,332 (Stark and Van Rijn 2010)	Liquid solution	Vegetables, fruits, herbs, plants, and mushroom substrates	*Water*[iv]. *pH adjustors:* hydrogen chloride[iv], sulfuric acid[iv], citric acid[iv], lactic acid[iv], sodium hydroxide[iv], potassium hydroxide[iv], ammonium hydroxide[iv]. *Solvents:* food grade solvent such as ethanol[iv] if for agricultural or food use. Other uses include many other solvents.
Valent BioSciences Corporation	0271158* (Huang, et al. 2016)	Liquid suspension concentrate	Fruits, mushrooms, pre- and post-harvest	*Water*[iv]. *Anionic surfactants*: polyelectrolyte polymers (such as sodium lignosulfonate[iv]), modified styrene acrylic polymers[N], polyoxyethylene sorbitan trioleates[iv], polyoxyethylene sorbitol hexaoleates[iv], dioctyl sodium sulfosuccinate[iv], sodium salts of naphthalene sulfonates[iii]. *Diluents:* glycerol[iv], hexylene glycol[iii], dipropylene glycol[iii] , polyethylene glycol[iv]. *Preservatives:* benzoates[N] and potassium sorbate[iv]. *Antifoams:* silicone based antifoam agents[N], vegetable oils[N], acetylenic glycols[N], and high molecular weight adducts of propylene oxide[N]. *Antifreeze:* ethylene glycol[iii] , 1,2-propylene glycol[iv], 1,3-propylene glycol[N], 1,2-butanediol[N], 1,3-butanediol[iii], 1,4-butanediol[iii], 1,4-pentanediol[N], 3-methyl-1,5-pentanediol[N], 2,3-dimethyl-2,3-butanediol[N], trimethylolpropane[iii], mannitol[iii], sorbitol[iv], glycerol[iv], pentaerythritol[iii], 1,4-cyclohexanedimethanol[N], xylenol[N], bisphenol A[N]. *Miscellaneous:* the patent application describes applying the product with an additional coating wax.
DSM IP Assets, B.V.	8,420,609 (De Haan and Van Rijn 2013); 9.615,581 (De Haan and Van Rijn 2017)	Needle-shaped crystals in aqueous suspension	Fruits, vegetables, and seed	*Water*. *pH adjustors*: hydrogen chloride[iv], benzoic acid[iv], propionic acid[iv], sorbic acid[iv], acetic acid[iv], lactic acid[iv], or sodium hydroxide[iv]. *Carriers:* fumed silica[iv]. *Solvents:* C1-C4 alcohols[N], glacial acetic acid[iv]. *Surfactants:* sodium lauryl sulfate[iv], dioctyl sulfosuccinate[iv], calcium chloride[iv], non-ionic surfactants[N]. *Thickening / bulking agents:* hydroxypropylmethylcellulose [iv] (HPMC), carrageenan[iv], methylcellulose[iv], xanthan gum[iv], gellan gum[iv], gum Arabic[iv].

Patent holding company	U.S. Patent Number (and source)	Product form	Uses	Formulants
N/A, referenced by Stark	N/A (Stark and Tan 2003).	Emulsion	Fruits	*Emulsifier:* lecithin[iv].

Key: * = Patent application only, not granted; [iii] = Present on 2004 EPA List 3; [iv] = Present on 2004 EPA List 4; [N] = Not able to confirm 2004 EPA list status.

Formulants used with natamycin for other purposes, such as in beverages, baked goods, cheese coatings, and other dairy products are outside the scope of this report.

Status

Historic Use

The discovery of natamycin was first reported in 1958 (Struyk, et al. 1957-1958). At that time, it was named "pimaricin," based on the location from which the bacteria that produced it was found in Pietermaritzburg, South Africa. Natamycin was again discovered independently in 1959, this time named "tennecitin," based on the location of the soil isolate, which came from Chattanooga, Tennessee (Burns 1959). Later, it was named "natamycin" by the World Health Organization (Brik 1994).

Natamycin is unique, in that as of 2003, it was the only microbially derived antifungal compound used as a food preservative (Stark and Tan 2003). In addition to its well-established uses as a food additive for preserving cheese, sausage, and other food products, natamycin was studied as a potential fungicide for fruit diseases as early as 1958 (Eckert 1967).

In the United States, natamycin has been approved for use in mushroom production by the EPA since 2012, and since 2016 for post-harvest fruit production (EPA 2017a). No data was found regarding how many producers use it, how often, or in what total quantities for any of the petitioned uses. Published EPA reviews of natamycin did not include numerical estimates of the cumulative quantity of natamycin that was expected to be used (EPA 2016b, EPA 2012a). Pennsylvania State College of Agricultural Sciences, which maintains a dedicated mushroom research facility and provides extension support for mushroom growers, does not include natamycin as a chemical control in guides or fact sheets (Penn State College of Agricultural Sciences, n.d.) (Beyer n.d.).

Organic Foods Production Act, USDA Final Rule:

Natamycin is not listed in the Organic Foods Production Act (OFPA) nor in the NOP regulations.

For use as an input in crop production, the NOP regulations permit nonsynthetic substances that are not otherwise prohibited by §205.602 of the National List. The NOP Handbook contains guidance documents that describe the procedures used for classifying materials as synthetic or nonsynthetic. The Organic Materials Review Institute (OMRI) has classified natamycin as nonsynthetic and previously included natamycin products on the OMRI Products List©. Under NOP regulations, OMRI currently considers natamycin as an issue beyond resolution, as indicated on the OMRI website: "Although OMRI has determined that natamycin is a nonsynthetic material based on the Draft NOP Guidance on Classification of Materials (NOP 5033),[4] the NOP has stated that this substance is not allowed under the NOP regulations and has instructed OMRI not to list products containing natamycin" (OMRI 2017). The Washington State Department of Agriculture (WSDA) Organic Food Program also does not currently include any natamycin-based fungicides on its publicly available approved organic inputs lists (WSDA Organic Program 2017)

[4] Since publication of the issue on OMRI's website, the final version of the NOP Guidance Classification of Materials has been published (USDA NOP 2016b).

Natamycin is prohibited for use in organic processing and handling because it is a nonorganic substance which is not included on the National List sections 205.605 or 205.606. In December 2005, natamycin was petitioned as a nonsynthetic nonagricultural substance for use in organic processing and handling, specifically for use as post-baking surface treatment of baked goods to prevent or delay growth of mold (George Weston Bakeries, Inc. 2005). The NOSB Handling subcommittee considered the petition in 2007. The subcommittee's recommendation identified natamycin as synthetic, and the motion to add the substance to §205.605(b) failed (NOSB Handling Subcommittee 2007). The full NOSB considered the petition at the spring 2007 meeting. The minutes from that meeting indicate that the board members were persuaded that natamycin is not synthetic.[5] The full board voted on a motion to list natamycin on §205.605(a) as a nonsynthetic and the motion failed.[6] At the time, the Board did not separately vote on the classification of natamycin as synthetic or nonsynthetic.

International

Canadian General Standards Board Permitted Substances List (CAN/CGSB-32.311-2015)
http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/lsp-psl-eng.html
"Biological organisms" (living, dead, or non-viable) are permitted for use as crop production aids and materials on Table 4.3 of CAN/CGSB-32.311-2015. Examples given in the listing include microbial organisms (*Bacillus thuringiensis*) and microbial products (spinosad). Natamycin itself is not a biological organism; however, it could be considered a microbial product much like spinosad.

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling, and Marketing of Organically Produced Foods (GL 32-1999)
http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1
http://www.codexalimentarius.org/download/standards/360/cxg_032e.pdf
The CODEX Alimentarius *Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods*, Annex 2, Table 2 (Substances for Plant Pest and Disease Control), III lists "Microorganisms used for biological pest controls" with the condition that the need for use be recognized by the certification body or authority. Specific products of microbial fermentation such as spinosad and fermented product from *Aspergillus* appear on the same table under section 1: Plant and Animal. Natamycin is not specifically listed in this section.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:250:0001:0084:EN:PDF
While microorganisms used for biological pest and disease control are permitted in Annex II of EC No. 889/2008, natamycin is not listed as one of the permitted substances produced by microorganisms in Annex II. Annex II is a closed list, and spinosad is the only microbially produced substance listed as allowed for pest control.

Japan Agricultural Standard (JAS) for Organic Production http://www.maff.go.jp/e/jas/specific/criteria_o.html
Natamycin is not specifically listed in JAS regulations. However, Notification No. 1605, Japanese Agricultural Standard for Organic Plants (JAS 2017), Article 5 lists substances for preparation and includes "Substances for preparation derived from microorganisms." Natamycin, while not itself a microorganism, is derived from microorganisms and therefore meets this definition.

[5] Excerpt from meeting transcript on March 28, 2007: "I think we've heard pretty compelling public comment yesterday and today and I think we are persuaded that natamycin is not in fact synthetic and so the prohibition for listing something for the purpose of being used as a preservative does not apply to a nonsynthetic."
[6] NOSB does not issue final recommendations for failed motions; there is no final recommendation to reference.

International Federation of Organic Agriculture Movements (IFOAM)
http://www.ifoam.bio/en/ifoam-norms
Bacterial preparations are listed as a permitted substance in Appendix 3: Crop Protectants and Growth Regulators. Natamycin is not specifically listed.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

Natamycin is a naturally occurring substance produced by bacteria, so an exemption from OFPA for a synthetic substance may not be applicable (see *Evaluation Question #3*, which suggests that natamycin may be classified as nonsynthetic based on NOP Guidance 5033-1). Natamycin inhibits spore germination and disrupts the normal function of membranes containing ergosterol. The EPA has not identified Natamycin as an inert (EPA 2017), but has approved its use as an active fungistat ingredient when used in enclosed mushroom growing facilities (EPA 2012a). Natamycin is exempted from the requirement of a tolerance for residues on fruits when used in post-harvest handling (EPA 2016a).

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Regardless of the application, natamycin production typically involves two primary steps: 1) biosynthesis of natamycin through submerged aerobic fermentation and 2) extraction and purification of natamycin from the post-fermentation broth through the use of solvents, pH/solubility adjustment, and/or physical means. Afterwards, natamycin may be formulated with other ingredients for end use. During these processes, the chemical structure of natamycin is not permanently changed. Depending on the solvents used, natamycin may form reversible intermediates that revert back to the original structure produced by bacteria, and it may gain or lose waters of hydration, depending on processing (such as when drying or producing solvates). Details of the chemical changes are described in *Evaluation Question #3*.

Biosynthesis of natamycin through fermentation
Natamycin occurs as a secondary metabolite in *Streptomyces spp.* and its production is positively affected by available oxygen (Beites, et al. 2011). As such, aerobic conditions are necessary for natamycin production. *Streptomyces spp.* are typically grown in submerged aerobic conditions in liquid growth media (Struyk, et al. 1957-1958) (Burns 1959) (Beites, et al. 2011) (Elsayed, Farid and Enshasy 2013). This process involves taking growth from a previous liquid culture, and using that to inoculate production volumes of liquid media. Growth media temperatures have been reported at 25°C for optimal production (Burns 1959), and 30°C (Elsayed, Farid and Enshasy 2013). Natamycin yield is reportedly optimal between pH 5.0 and 6.5 if maintained by pH control agents (Eisenschink and Olson 1993).

Eisenschink (1993) describes in detail a process for biosynthesizing natamycin. *Streptomyces sp.* spore suspensions are prepared and serially propagated until finally transferring to an 80,000 liter production fermentor. During fermentation, media is aerated through agitation or injection of sterile air in order to maintain a dissolved oxygen level of 20 to 80 percent. Components of the production (growth) media

include sources of nitrogen, carbon, vitamins, inorganic elements, and trace elements. Depletion of the carbon source negatively impacts natamycin yield, so it is added continually during production. The carbon source is discontinued prior to the completion of fermentation so that little to no carbon source is left at the termination of production. Antifoaming agents (such as silicone-based products) are added as needed. During fermentation, the pH of the production media decreases. Alkaline and other pH adjusting materials are added to increase and maintain the pH within the optimum range (such as sodium, potassium, or calcium hydroxides, along with sodium and potassium citrates). Growth proceeds through three phases: during the first phase, *Streptomyces sp.* increases, and natamycin increases exponentially. In the second phase, natamycin production continues, but linearly. In the final phase, natamycin concentration plateaus.

Improvements in natamycin growth media have led to decreases in the time to reach peak production. When Burns reported on natamycin in 1959, peak production occurred approximately 96 hours after inoculation (Burns 1959)(Table 3). In 2013, Elsayed et al. found that adding acetic and propionic acid to the growth medium in a 7:1 ratio yielded a 250 percent increase in natamycin production, with a decrease in production time from 96 to 84 hours (Elsayed, Farid and Enshasy 2013). Other nutrients may be used in growth media, such as ammonium sulfate or sodium nitrate, but these substances were not specifically mentioned in the literature.

The petition does not include specific details about the medium or technique used for biosynthesis. However, DSM has reported using a submerged aerobic fermentation method of production in the past (DSM Food Specialties Inc. 2015), and the European Food Safety Authority report included with the petition corroborates the use of this technique (Technology Sciences Group, Inc. 2016), and some information about DSM's growth media can be ascertained from their 2015 FDA GRAS notice (see Table 3).

Table 3: Natamycin growth media components

Source	Type	Components
(Struyk, et al. 1957-1958)	Experimental	Soybean meal, glucose, nutrient salts.
(Burns 1959)	Experimental	Peptone, phytone, beef extract, yeast extract, and glycerol. Inositol dextrin, and galactose were satisfactory replacements for glycerol as a carbohydrate source.
(Eisenschink and Olson 1993)	Patent	Difco "Bacto" peptone, Hormel peptone PSR 5, corn steep liquor, sodium chloride, glucose.
(Eisenschink, Millis and Olson 1997)	Patent	Carbon sources such as glucose, polysaccharides, and corn or potato starches. Non-yeast and yeast protein in a 3:1 to 9:1 ratio. Non-yeast protein sources include soy protein isolates, flours, or meals; or beef extract or protein hydrolysates. Yeast protein sources include extracts, autolysates, etc. Vitamins, inorganic elements and trace minerals: potassium, sodium calcium, boron, iron, copper zinc, etc. (undisclosed forms)
(Elsayed, Farid and Enshasy 2013)	Experimental	Glucose, beef extract, yeast extract, asparagine, and monopotassium phosphate, sodium acetate, and the sodium salt of propionic acid.
(DSM Food Specialties Inc. 2015)	Production	Undisclosed soy carbon source, inorganic salts, lye solution for pH control.

Extraction and purification

At the end of fermentation, the post-fermentation broth contains natamycin and various undesirable by-products of the fermentation process, such as biomass solids (bacterial mycelium), dissolved or suspended

nutrients, other fermentation products, and water (Raghoenath and Webbers 2000). Different strategies are used to extract and purify natamycin from the post-fermentation broth. Approaches for isolation of natamycin initially involved using organic solvents to isolate natamycin and adding low solubility liquids to create a precipitate (Struyk, et al. 1957-1958) (Burns 1959). More recent processes involve pH adjustments to recover natamycin, or using solubility enhancing salts and dilution (Eisenschink, Millis and Olson 1997) (Olson, Millis and Reimer 1997). Other current strategies omit the use of organic solvents, and instead rely on isolation through particle size and density sorting (Raghoenath and Webbers 2000). This section describes the evolution of natamycin processing, culminating in the petitioner's process.

Struyk and Burns relied on initially filtering, then moving natamycin into an alcohol solvent, and then forcing precipitation through the addition of a low solubility material (Struyk, et al. 1957-1958) (Burns 1959). Struyk used organic solvents such as formamide, and then water to precipitate natamycin, while Burns used n-butanol as the solvent, created a highly saturated solution through evaporation, and then added cold ether to precipitate natamycin. Struyk further purified natamycin by re-dissolving the crystals in hot methanol, followed by filtration and precipitation in water.

Cultor Food Science, Inc. patented a method whereby the broth culture pH level was adjusted with a base to 10 or 11 (Eisenschink, Millis and Olson 1997). Then, a water miscible solvent (preferably isopropanol) was added to further solubilize natamycin, followed by filtration to remove solids (mycelium). The solids were washed with additional solvent to extract additional residual natamycin. The pH of the solution was lowered with an acid (such as hydrochloric acid) to cause precipitation of natamycin, and then the crystals were subsequently isolated through filtration, washing with a water-isopropanol mixture, and evaporated or spray dried (Eisenschink, Millis and Olson 1997).

Biotechnical Resources L.P. patented a continuous flow process for the recovery of natamycin using methanol (Olson, Millis and Reimer 1997). Cool methanol was added to the broth, preferably at 15°C. The mixture was then pH adjusted to between 1 and 4.5 for 30 minutes to 30 hours. Alternatively, no pH adjustment was performed and instead, a solubility enhancing salt was added, such as calcium chloride. Solids were removed by filtration or centrifugation, and the pH of the solution was raised to between 6 and 9 with sodium hydroxide to precipitate natamycin crystals, unless a solubility enhancing salt had been added, in which case water was added to precipitate the crystals. The crystals were further washed and dried to increase the purity (Olson, Millis and Reimer 1997).

Gist-Brocades B.V. patented an isolation process in 2000 which omitted the use of organic solvents as the primary means of recovery (Raghoenath and Webbers 2000). Instead, biomass was first disintegrated using a variety of possible methods, preferably heat and pH treatment, and then natamycin crystals were isolated through gravity separation. Disintegration of the biomass took place for 1-8 hours preferably at 30-35°C, with sodium hydroxide, ammonium hydroxide, or potassium hydroxide being used to adjust the pH level to between 8 and 10, followed by neutralization with hydrochloric acid, phosphoric acid, sulfuric acid, or acetic acid. Neutralization preferably occurred after separation of natamycin from the broth. Other disintegration methods were covered by the patent, such as physical, enzymatic, and surface active chemical methods. Enzymatic treatments involved incubating cell wall and organic polymer decomposing enzymes such as lysozyme, xylanase, cellulose, protease, glucanase, lipase, and amylase. Disintegration with surface active agents included octylphenoxypolyethoxyethanol compounds, for example Triton X-100 for 1-24 hours. Separation of the larger natamycin crystals from the smaller disintegrates in the broth was accomplished using an upflow column or hydrocyclone, with additional water and sodium chloride added as necessary. The purity and yield were adjustable with this method, being able to produce an approximately 90 percent pure (anhydrous basis) natamycin product (Raghoenath and Webbers 2000).

Gist-Brocades also patented a process to make novel natamycin crystal forms claimed to have increased bioactivity (van Rijn, et al. 1998). Crystals of alpha-natamycin were dissolved in methanol, and then the solvent was evaporated under vacuum leaving a unique natamycin crystal form, called delta-natamycin. Delta-natamycin could also be hydrated in a 76 percent relative humidity environment to form the trihydrate gamma-natamycin with yet another crystal structure. Additionally, the patent described the preparation of natamycin salts (such as calcium and barium). These processes involved passing nitrogen

gas was passed through a saturated solution of calcium or barium hydroxide in water and adding natamycin. The resultant crystals were filtered and washed with water and acetone, then dried (van Rijn, et al. 1998).

The petitioner describes using heat to lyse the biomass, consistent with the initial process described in the 2000 Gist-Brocades patent[7] (but not necessarily subsequent steps). The mixture is then centrifuged to separate the biomass from the broth medium containing the natamycin crystals. DSM states that a solvent is added during this process to maintain microbiological stability. Based on a flow chart submitted to the EPA, the solvent may be n-propanol (DSM Food Specialties Inc. 2015). A pH adjusting process is used to precipitate the natamycin crystals from the broth, possibly using lye (sodium or potassium hydroxide) as one of the pH adjustors. The crystals are pressed in order to remove the solvent and excess water (Technology Sciences Group, Inc. 2016). In the aforementioned manufacturing process flow chart submitted to the EPA, the petitioner shows an additional resuspension of crystals in n-propanol and water, followed by washing, filtering, and drying.

DSM additionally patented a process whereby natamycin crystals are dissolved in an alkaline water solution with a pH level between 11.0 and 13.0 using sodium hydroxide (De Haan and Van Rijn 2013). The solution is then neutralized to a pH between 6.0 and 8.0 using hydrochloric acid, whereby natamycin crystals with a needle shape (as opposed to plate shape) form over a period of 10-30 minutes and at a temperature between 15-25°C. The crystals can then be dried or left in solution. According to the patent, the needle shaped crystals are advantageous when making natamycin suspensions (De Haan and Van Rijn 2013).

Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Natamycin is commercially manufactured through biosynthesis, extraction, and purification as described in Evaluation Question #2. Biosynthesis of natamycin through fermentation is a naturally occurring biological process. NOP Guidance 5033, Classification of Materials, states at §4.7 that products of naturally occurring biological processes, such as fermentation are statutorily considered natural and nonsynthetic (USDA NOP 2016b). During the extraction and purification steps to recover natamycin from the post-fermentation broth, synthetic extractants may be used and temporary chemical changes may occur; however, the resulting natamycin substance is not chemically changed from the original substance that was produced by fermentation. NOP Guidance 5033 §4.6 states that nonorganic materials may be extracted with solvents, acid-base extraction, and physical methods such as filtration, crushing, centrifugation, and gravity separation (USDA NOP 2016b). Extraction techniques must meet three criteria in order for the extracted material to be considered nonsynthetic. Natamycin is evaluated against the decision tree in NOP Guidance 5033-1 below.

To further evaluate natamycin as described in Evaluation Question #2 against NOP Guidance 5033-1 (USDA NOP 2016a):

- *Is the substance manufactured, produced, or extracted from a natural source?(Box 1)*
  Natamycin is produced by a biological mediation of substrates via aerobic fermentation with *Streptomyces ssp.*).

- *At the end of the extraction process, does the substance meet all of the criteria described at §4.6 of NOP 5033?(Box 2b)*

  - *At the end of the extraction process, the material has not been transformed into a different substance via chemical change;*

[7] Gist-Brocades B.V. was purchased by DSM's parent company in 1998. The patent mentioned here was originally filed by Gist-Brocades in 1997.

The extraction methods used to isolate natamycin involve either physical processes, or processes that take advantage of natamycin's low solubility in solvents such as water, and relatively high solubility in other solvents such as methanol or at pH extremes. These processes do not permanently chemically alter natamycin. Some impurities may be formed incidentally, such as 13-hydroxy-2,4,6,8,10-tetradeca pentane-1-al (Brik 1976).

- *The material has not been altered into a form that does not occur in nature;*
  No information was found that elucidates under what circumstances natamycin is produced by *Streptomyces spp.* in nature, or if it is produced in sufficient quantity to form crystals. If natamycin were produced by *Streptomyces spp.* in the soil, there is no reason to believe it would differ from that produced in the methods described within this report.

- *Any synthetic materials used to separate, isolate, or extract the substance have been removed from the final substance (e.g., via evaporation, distillation, precipitation, or other means) such that they have no technical or functional effect in the final product.*
  Natamycin forms solid crystals which precipitate out of solution during the extraction process. Solvents and other materials used in processing are separated through physical means such as filtration, washing, and evaporation. A residual amount of solvents and other materials may remain, but are not considered to have a technical or functional effect in the final product.

- *Has the substance undergone a chemical change so that it is chemically or structurally different than how it naturally occurs in the source material?(Box 2)*
  Based on the information described above in 2b, natamycin does not undergo a chemical change so that it is chemically or structurally different. Other materials that have similar extraction and purification techniques have been classified as nonsynthetic, including citric acid and glucono delta-lactone, both classified as nonsynthetic on §205.605(a).

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

Application rates

As natamycin is effective at low concentrations, application rates are small. For the petitioned use in mushroom production, a maximum application rate of 0.65oz of natamycin (the technical grade of the active ingredient [TGAI]) per 1000 ft^2 is used (Technology Sciences Group, Inc. 2016). For post-harvest use in fruit production, labels for products with EPA Reg. No. 87485-2 give various application rates. For in-line aqueous applications, 28 to 114 fluid ounces of formulated end-product (10.34 percent natamycin TGAI) can cover 50,000 to 200,000 pounds of fruit, depending on target crop and disease. Application methods such as drenching and flooding use 57 to 114 fluid ounces per 100 gallons of water though it is not clear how many pounds of fruit this covers. Labels for products with EPA Reg. No. 87485-3 (for use on pineapples) show an application rate of 4 to 32 fluid ounces per gallon of water and aqueous dilution of wax, with 0.034 fluid ounces of this dilution applied to the peduncle (stem). Based on maximum label use rates for in-line flood applications (EPA Reg. No. 87485-2), natamycin is applied at 16mg/kg fruit.

Post-application residues on crops and in the environment

Residues remaining after application are low. In a crop field trial submitted for review to the EPA, maximum residues on unwashed mushrooms were 0.2370 mg/kg (Jones 2011). No crop study data was found regarding residues on fruits treated with natamycin post-harvest. As mentioned earlier in the *Approved Legal Use of Substance* section, natamycin is exempt from the requirement of a residue tolerance when used in accordance with label directions and good agricultural practices for post-harvest treatment on the following raw agricultural products: mushrooms, pineapples, citrus, pome, stone fruit crop groups, avocado, kiwi, mango, and pomegranates (EPA 2016a).

Water used to apply natamycin to fruit or that is leached from mushroom production may be one of the more likely sources for residues entering the environment, although information on this potential was not available in the literature. Other potential sources include residuals from natamycin-treated food products that enter the waste stream, and consumed food products that may pass through the digestive tract. The Joint FAO/WHO Expert Committee on Food Additives concluded that natamycin is minimally absorbed during digestion and is primarily excreted in the feces (WHO 2002). Therefore, if natamycin is still present on food products at the time of consumption, it may be possible that human sewage contributes to natamycin residues in the environment.

The manner in which enclosed mushroom production occurs limits the accumulation of natamycin and its breakdown products within mushroom substrates. As mushrooms are grown, they deplete their substrates, which must be entirely replaced (Munshi, et al. 2010). Spent mushroom substrates may go on to be used as soil amendments or compost feedstocks. Natamycin products registered for use on mushrooms are currently limited to EPA Reg. No. 87485-2, and contain label use instructions that direct users to steam spent substrate for at least 12 hours at 65°C or greater prior to disposal. Natamycin is stable above 100°C at neutral pH, and therefore would theoretically not break down by the steam treatment prescribed. In a field trial reviewed by the EPA, natamycin residues were not detected[8] in mushroom substrates after steam sterilization (Jones 2011). The fate of the natamycin (whether it was broken down by the treatment or otherwise removed) was not disclosed in the study.

After post-harvest processing, crops may be taken directly to market, refrigerated, or placed in controlled atmosphere storage. Natamycin, if protected from UV light, is stable in such conditions. The length of time that natamycin residues remain active likely depends on the presence of UV light, or whether formulants or packaging are used that protect natamycin. Due to its thermal stability, temperature is unlikely a factor in the length of time natamycin remains intact on fruit surfaces. Uneaten fruit that is disposed could theoretically create an avenue for minor amounts of natamycin to reach the environment.

Decomposition / degradation

Some information regarding the decomposition of natamycin is known, but a complete picture is far from evident. Much of the available information on its decomposition is based on applications of various wavelengths of light (Struyk, et al. 1957-1958) (Burns 1959) (Brik 1976) (Koontz, et al. 2003), solvents (Brik 1976), heat (Struyk, et al. 1957-1958) (Burns 1959), and pH extremes in a laboratory setting (Brik 1976) (Burns 1959) (Brik 1994). These studies do not necessarily reflect what happens to natamycin in the environment. Furthermore, studies have often focused on what inactivates natamycin (eliminating functionality), rather than its decomposition products. Studies that have investigated the decomposition of natamycin, such as performed by Brik (1976), do not identify how the decomposition products themselves would be further broken down, or whether they would be metabolized by native organisms in the environment.

Natamycin degrades in the presence of: ultraviolet (UV) light (Koontz, et al. 2003); oxidants such as peroxides, chlorine, and heavy metals (EFSA 2009); and pH extremes (Brik 1976). A 20 mg/L aqueous solution of natamycin without UV protectants was degraded within 24 hours when exposed to fluorescent lighting, such as that found in deli cases (Koontz, et al. 2003). Degradation does not involve complete molecular decomposition, but rather a loss of function or biological activity. When degraded with UV light, the primary change is that the polyene moiety loses a double bond, becoming a triene (Brik 1976). Oxidation also presents stability issues for natamycin. In one study, when applied to cucumber leaves, natamycin lost most of its activity within 3 hours in darkness due to autoxidation; however, it is not clear what form of natamycin was used (anhydrous or trihydrate) (Dekker 1963). Breakdown in the presence of acids creates free mycosamine and dimers (pairs) of natamycin and modified lactone rings much larger than natamycin itself (Brik 1976). Alkaline environments can hydrolyze the lactone ring, producing a non-cyclic aldehyde, while other parts of the ring can break down into acetone and acetaldehyde (Brik 1994). The EPA reports that natamycin is degraded by metals and metal ions, but the decomposition products are not mentioned (Jones 2011).

[8] With a limit of quantitation (LoQ) of 0.1mg/kg (ppm).

Natamycin can be UV- and/or oxidation stabilized by the addition of substances such as ascorbic acid (Burns 1959), plant juices (Dekker 1963), chlorophyll (Brik 1981), and sodium potassium chlorophyllin (Koontz, et al. 2003). Additionally, packaging or any other substance that absorbs light between 300 and 400nm will protect natamycin from photodegradation. Components of carnauba wax (used to coat fruit) have been shown to absorb UV light in the 250 to 350nm range (Freitas, et al. 2016). In black olives, application of 100mg/L of natamycin to brines suppressed fungal growth for the length of the trial (60 days) at room temperature. Quantification of natamycin present in the brine at the end of the trial was not evaluated, and it is not known what UV stabilizers may have been present (Hondrodimou, Kourkoutas and Panagou 2011).

Accumulation / biological fate

Information regarding the persistence, accumulation, or concentration of natamycin in the environment is not available in the literature. Natamycin has very low solubility in water, and therefore it is unlikely to build up in aquatic environments though may be incorporated into sediments if not broken down. In shallow or clear aquatic environments subject to sunlight, there is potential for natamycin to degrade due to its sensitivity to the UV spectrum, as discussed above.

While detailed information was limited with respect to natamycin, some biological fate data is present for nystatin, which shares physical and chemical similarities with natamycin. Nystatin lacks an epoxide ring which is present in natamycin (Figure 1, III), and its macrolide ring contains 38 members instead of natamycin's 26 (U.S. National Library of Medicine 2017b). Otherwise, nystatin is a tetraene macrolide antimycotic, containing mycosamine. Nystatin in the air has a half-life of 1.5 hours due to degradation by hydroxyl radicals; 2.6 hours due to ozone; and an unknown half-life due to photolysis by sunlight (U.S. National Library of Medicine 2006). A closed bottle test indicated that biodegradation (biological means) was slow for nystatin, and not an important environmental fate process. Bioconcentration in aquatic organisms was low, with a bioconcentration factor (BCF) value of 22; a material is not considered to pose a risk for bioconcentration until reaching a value of 1000 (Arnot and Gobas 2006).

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Natamycin inhibits spore germination and disrupts the normal function of membranes containing ergosterol, for which the EPA describes as a "non-toxic" mode of action (EPA 2016c). The EPA considers lethal, but non-toxic pesticides to include suffocating agents (oils), desiccants, and abrasives; in other words, materials that are not poisonous to the target organism (Leahy, et al. 2014).

Natamycin has low to moderate oral toxicity, depending on the animal (EFSA 2009). The European Food Safety Authority reported the oral LD50 in male rats was 2700 mg/kg, and 4700 mg/kg in females. The oral LD50 in mice was 1400 mg/kg, and 450 mg/kg for female guinea pigs. The No-Observed-Adverse-Effect Level (NOAEL) for rats in subchronic studies was 45 mg/kg of body weight per day.

A description of the toxicity mechanism was not found in published literature. Based on oral acute toxicity data, the EPA has classified it as category III (slightly toxic) (EPA 2012a). The EPA noted that no significant acute, subchronic, genotypic, developmental, or endocrinologic mammalian toxicity effects were observed, and toxicological endpoints were not identified (EPA 2016c). See *Evaluation Question #10* for more information on human toxicity.

Information regarding the breakdown products of natamycin under natural environmental conditions is not available in the published literature. However, in laboratory conditions under acidic or basic extremes, natamycin was found to decompose into mycosamine, acetone, aldehydes, acetaldehyde, ammonia, and various macrolide ring structures (e.g., aponatamycin) (Brik 1981). The median lethal dose (LD50) for mice

ranged from 150 to 600 mg/kg of body weight when treated via intraperitoneal injection[9] with decomposition products of natamycin (FDA 2015).

Although the decomposition products of natamycin under natural circumstances are not described in literature, the potential toxicity of the experimentally derived decomposition products is explored in the following paragraphs.

Mycosamine

Brik (1981) noted that the products of acid, alkaline, and UV-treated natamycin such as aponatamycin (one of the macrolides) and mycosamine are less toxic than the parent compound, but the animals tested or the method of application were not disclosed.

Acetone

Acetone is a naturally occurring ketone in the body, which can be metabolized for energy. Acetone has low toxicity with an oral LD50 values for adult rats of 5800-7138 mg/kg (U.S. National Library of Medicine 2015b). Values as high as this are extremely unlikely to occur through use of natamycin due to both the application rates involved, and through microbial oxidation of acetone by soil bacteria (Taylor, et al. 1980).

Aldehydes

Aldehydes are pervasive in the environment, and many have documented health risks (LoPachin and Gavin 2014). With the exception of acetaldehyde, no specific information is available for the forms of aldehydes created from the decomposition of natamycin. Acetaldehyde is very soluble in water, and also binds to soil or suspended particles. It is broken down by microorganisms and is not expected to build up in aquatic organisms. At concentrations of 0.1 percent, it can induce mutations in nematodes, and is expected to be a carcinogen, based on animal studies. It has an oral LD50 in rats of 1930 mg/kg (U.S. National Library of Medicine 2015a).

Ammonia

Ammonia is highly reactive, and can volatilize, adsorb to soil, be metabolized by microorganisms, or be taken in by plants. Ammonia is moderately toxic, with an oral LD50 in rats of 350 mg/kg. Concentrations of this amount due to the application of natamycin are extremely unlikely, based on application rates and reactivity (U.S. National Library of Medicine 2016).

Evaluation Question #6: Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

No literature from the EPA, FDA, National Institute of Environmental Health (NIEHS), the European Environment Agency (EEA), or from academic or independent papers was found that directly related to environmental contamination from the production, use, misuse, or disposal of natamycin. The EPA did not require Tier 1 studies to assess ecological hazards, environmental fate, groundwater data, or endangered species assessment prior to registration of natamycin (EPA 2012a). Furthermore, no published information could be found directly related to pollution created from the production of secondary metabolites by bacteria. An EEA report from 2010 noted that very little data on the environmental exposures, fate, and impact of pharmaceutical products in the environment exist (EEA 2010).

In the biosynthesis of natamycin, wastewater containing spent growth media, bacterial mycelium, pH adjusters, antifoaming agents, and other materials may be created. Wastewater treatment plants do not remove micro-pollutants completely (Martz 2012). Other metabolites or chemicals may be present in such wastewater, and if not treated properly, these materials may be emitted to the environment. Once released natamycin could migrate into sediments, but would be unlikely to bioconcentrate in aquatic organisms, based on similarities to nystatin as discussed in *Evaluation Question #4*.

[9] Intraperitoneal (IP) injection is the injection of a substance into the peritoneum (body cavity).

Misuse of the product, such as application at higher rates than approved by the EPA, would be unlikely to affect the surrounding environment due to the restricted locations that it is used (e.g., enclosed mushroom facilities, or in facilities post-harvest). Application to non-approved agricultural crops could negatively affect germination of other fungi, including beneficial fungi such as *Paecilomyces* and *Trichoderma sp.* (Brothers and Wyatt 2000).

Evaluation Question #7: Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

Safety data sheets (SDS) indicate that natamycin products with EPA Reg. Nos. 87485-1, and -2 are chemically stable. An SDS for EPA Reg No. 87845-3 cannot be located using publically available resources. Specific chemical interactions are not known to occur beyond those described within manufacturing processes noted in *Evaluation Question* #2, with the exception that it is degraded by metal or metal ions (Jones 2011). Natamycin may be formulated with other inert ingredients (as described in *Combinations of the Substance),* but the specific identities of these materials are not publicly available. Natamycin may dissolve in some solvents, or break down in the presence of strong acids or bases. No information was found showing that natamycin is used as a precursor or a feedstock for production of other chemicals, whether used in organic crop production or otherwise.

Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

Natamycin used as petitioned is unlikely to significantly affect the agro-ecosystem due to its mode of action and because it is applied in post-harvest or enclosed mushroom facilities. As petitioned, natamycin would not be applied to soils directly (although it may be indirectly applied via spent mushroom media as a soil amendment). Furthermore, natamycin is not expected to have a direct effect on earthworms, mites, grubs, bacteria, nematodes, or algae, unless applied at very high dosages as ergosterol does not play a significant role in animal, plant, and bacterial membranes (Dupont, et al. 2012) (Sáenz, et al. 2012). It can affect protozoa and fungi; however, as petitioned it would not be applied to the soil, and could only affect them through mishandling or misapplication. It is not expected to affect soil temperature, water availability, pH, nutrient availability, salt concentration, solubility, or other soil physicochemical parameters. As petitioned, natamycin would be unlikely to affect plant-fungi dynamics in the soil, such as mycorrhizal relationships, because it is not applied to growing plants or the soil.

The EPA determined that based on its use in mushroom production, natamycin exposure to non-target organisms was not expected; however, they did not pursue environmental fate data, and assumed that it would solely be used indoors. The EPA did not identify any toxic endpoints, and natamycin presented little if any risk to nontarget organisms (EPA 2012a).

Potential for fungal resistance to natamycin

The specific petitioned uses have only been approved in the United States since 2012 (mushroom production) and 2016 (post-harvest); long term evaluations of resistance due to the use of natamycin as petitioned were not identified. Looking beyond the petitioned use, the European Food Safety Authority (EFSA) believed that there was a potential risk of the development of resistant fungi when natamycin was used as a food additive, but that the risk and level of resistance would be low (EFSA 2009). EFSA reported that studies conducted in cheese warehouses and dry sausage factories have not shown a change in the fungal flora during 10 years of natamycin application.

Numerous studies show that resistance to natamycin can be induced in the laboratory. Resistance to natamycin by fungi such as *Cryptococcus neoformans, Aspergillus fennelliae,* and *Candida albicans* has been induced *in vitro* since at least the 1970s (Kim and Kwon-Chung 1974) (S. Kim, J. Kwon-Chung, et al. 1975)

(DSM Food Specialties Inc. 2015) and earlier for other polyenes such as amphotericin B (Hebeka and Solotorovsky 1965). Resistance by fungi to natamycin has typically come at a fitness cost, with a loss or reduction of virulence, asexual reproduction, sexual reproduction, growth rate, and thermal tolerance. Increased resistance was associated with changes in biosynthesis of ergosterol or ergosterol-like sterols. More recently, 20 fungal isolates, most different species, were evaluated for resistance in a laboratory setting using incrementally increasing concentrations of natamycin. Resistance was induced in 13 of the 20 isolates, with *Aspergillus ochraceus* also showing a threefold increased resistance to amphotericin B and nystatin (Streekstra, Verkennis, et al. 2016). When natamycin was removed, most strains with increased tolerance showed reduced growth, but not all; *Aspergillus terreus, Colletotrichum musae,* and *Geotrichum candidum* showed changes in appearance, but not colony size. Other fitness parameters apart from colony growth rate were not evaluated. In another study, of 319 strains of yeast taken from inflamed cow udders, 40.8 percent were resistant to natamycin (Lassa and Malinowski 2007); however, this data was not compared to any previous analysis and so no conclusions regarding the acquisition of resistance can be made.

At the March 2017 meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Russian Federation requested a safety re-assessment of natamycin for the Codex Committee of Food Additives to determine whether natamycin should remain on the General Standard for Food Additives (GSFA) list. The request referenced emerging data about the role of natamycin in promoting antimicrobial resistance and speeding up virulence and pathogenic potential of microorganisms that cause food-borne illness, as well as its effect on the misbalance of microflora in the gut, immunity status and other functions in the human body (CCFA 2017a). The referenced data was not included in the published meeting materials. The Egypt delegation questioned the proposed deletion of natamycin from the GSFA as being contrary to the CCFA procedures and opposed such a move due to the technological usage of natamycin under the approved safe limits (CCFA 2017b). However, the Committee agreed to obtain scientific advice and information is expected in December 2017 (CCFA 2017c).

The manner of application of natamycin as petitioned isolates both the antimycotic, and the population of fungi exposed to it. According to Anderson (2005), drug resistant phenotypes in fungi usually remain locally isolated and do not disseminate back into the larger population, unless there is a general advantage to the larger population (Anderson 2005). So far, natamycin resistant strains have been mostly (but not entirely) associated with reduced fitness (S. Kim, J. Kwon-Chung, et al. 1975) (Streekstra, Verkennis, et al. 2016), and therefore selection pressure would be low unless regularly exposed to natamycin. As natamycin is used more widely, selection pressures may increase, but to what extent is not clear.

Potential for horizontal gene transfer resistance

Horizontal gene transfer (HGT) is the exchange of genetic material between strains or species, as opposed to vertical exchange between parent and offspring within species. HGT primarily occurs in prokaryotes (such as bacteria). Recently, HGT has been identified in eukaryotes, though more barriers to its occurrence exist and the rate of transfer is low, based on current analyses (Ku, et al. 2015) (McInerney 2017). Identifiable HGT events themselves are typically not recent, having occurred in distant evolutionary history. It is thought that when HGT does occur in eukaryotes such as fungi, the other partner is more often a bacterium, though not always (Fitzpatrick 2012). Due to natamycin's mode of action, acquisition of direct resistance through HGT is difficult. While bacteria can carry resistance genes to the antibiotics that they produce (Jiang, et al. 2017), actinomycetes (such as *Streptomyces*) do not carry antimycotic resistance genes as the bacteria do not have the target molecule (such as ergosterol) in the first place (Seipke, et al. 2012). Therefore, HGT of resistance between bacteria and fungi is unlikely.

Examples of fungal-fungal HGT events do exist, including gene clusters encoding toxins such as fumonisin, to transfer of multiple complete chromosomes (Fitzpatrick 2012). Dalhoff and Levy state that fungal-fungal HGT has led *Candida spp.* and *Aspergillus fumigatus* to produce biofilms and gain resistance to polyene antimycotics (Dalhoff and Levy 2015). Biofilms and polyene resistance are known to occur in both *Candida* (Nett, et al. 2010) and *Aspergillus spp.* (Krappmann and Ramage 2013), and biofilms are associated with polyene resistance, but the acquisition by these species of those traits through HGT as Dahloff and

Levy suggest could not be confirmed in other publications. No documented direct resistance due to HGT could be found for the polyene antimycotics natamycin, amphotericin B, nystatin, or rimocidin.

Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

When used as petitioned, natamycin is unlikely to be harmful to the environment. If label instructions are followed, it is not applied to crops growing directly in soil. It has low toxicity to humans and other animals, and is not used at concentrations that would create a risk of acute exposure. Native fungi and protozoa in the agro-ecosystem are unlikely to be exposed to natamycin, except potentially through disposal of waste water. As natamycin activity is degraded by UV light and oxidants, the bioactivity of natamycin, once released, is likely to be low (unless the natamycin product has been formulated with stabilizers and is insufficiently diluted). While the environmental fate and breakdown products are not well documented, the known substances are unlikely to be harmful at the recommended application rates. Based on available data, fungal resistance to natamycin has yet to occur in a significant way, as discussed in *Evaluation Question #8*.

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Natamycin's exemption from the requirement for a tolerance of pesticide residue on food is based on the EPA's determination that there is a reasonable certainty that no harm will result from aggregate exposure to natamycin residues when used according to product labeling. The EPA evaluates pesticides by looking at toxicity of the substance as well as expected exposure through food and drinking water. Under these considerations, the EPA categorized natamycin as a Toxicity Category IV[10] active ingredient (EPA 2012b). Natamycin was found to have an acute oral toxicity of LD_{50}[11] > 3,000 mg/kg (Toxicity Category III), acute dermal toxicity of LD_{50} > 5,050 mg/kg (Toxicity Category IV), acute inhalation toxicity of LC_{50} > 2.39 mg/L (Toxicology Category IV), and primary eye irritation was severely irritating but with no positive effects after 24 hours (Toxicity Category III); Primary Dermal Irritation was slightly irritating (Toxicity Category IV). Natamycin is not a contact dermal sensitizer, is not a mutagen and is not cytotoxic (EPA 2016b) (EPA 2012a).

The JECFA established an allowed daily intake (ADI) for natamycin of 0-0.3 mg/kg of body weight in 1976. Human studies had shown no toxicological effects at a level of 3 mg/kg body weight per day, and an uncertainty factor of 10 was further included to calculate the ADI. The European Food Safety Authority (EFSA) estimated that the highest levels of human exposure to natamycin via food additive applications on cheese and sausage would be below the ADI, at 0.1 mg/kg body weight per day for children and below 0.05 mg/kg body weight per day for adults (EFSA 2009). At the time the ADI was established the JECFA also concluded that natamycin is poorly absorbed in the gut, and is primarily excreted in feces (JECFA 1976). The Committee considered additional studies in 2002 and reconfirmed the ADI.

In 2009 the EFSA published a review of natamycin's safety as a food additive. The report cited numerous animal tests which identified No-Observed-Adverse-Effect Levels (NOAELs) for natamycin in rats and dogs. These levels, all above the ADI, ranged from 45 to 6.25 mg/kg body weight per day for adverse effects such as decreased food intake, diarrhea, decreased body weight, and in one study, obesity. The EFSA reported no concerns for genotoxicity of natamycin, and rat tests evaluating reproductive toxicity resulted in a NOAEL of 50 mg/kg body weight per day (EFSA 2009).

[10] Toxicity Categories are defined at 40 CFR 156.62. Toxicity Category I indicates the highest level of toxicity. Category III indicates low toxicity and Category IV, the lowest toxicity.

[11] Lethal Dose $(LD)_{50}$ is the amount of a material, given all at once, which causes the death of 50 percent of a group of test animals.

The JECFA report from the 2002 meeting acknowledged that use of natamycin as an antifungal agent in food would result in exposure of intestinal microflora to its residues. However, the Committee speculated that because fungi are much less abundant in the human gastrointestinal tract than bacteria, and bacteria are not affected by polyenes, the consequences of indigenous microflora exposure to natamycin in the gut would be minimal (WHO 2002). One concern regarding microbial exposure to natamycin is the potential for development of resistance. Studies supporting the JECFA conclusion included surveys of cheese and sausage factories where natamycin has been used as a preservative. No change in composition or sensitivity of contaminating fungi to natamycin was found with the exception of one yeast strain in one of the studies. The authors reportedly found no yeasts or molds that were resistant to natamycin after several years of natamycin use (De Boer and Stolk-Horsthuis 1977). The authors also attempted to develop fungal strains resistant to natamycin under laboratory conditions by exposure to increased concentrations over 25-30 transfers. After 25 passes, *Candida albicans* was minimally less sensitive to natamycin, with 12-50µg/ml needed to induce sensitivity rather than the initial concentration of 2.5-12µg/ml. The resistant strains were reported to have reduced metabolic and growth rates and reverted to normal growth, metabolism and sensitivity to natamycin after polyene exposure had stopped (De Boer and Stolk-Horsthuis 1977) (WHO 2002). Reasons cited for the lack of development of fungal resistance to natamycin when used as a food additive include its environmental instability and its lethal antifungal activity (Delves-Broughton, et al. 2005).

Not all of the literature agrees on the absence of risk for the development of fungal resistance to natamycin and, by extension, to other antifungal polyenes, particularly those with importance as medical treatments. Dalhoff and Levy (2015) describe how applications of natamycin in yogurt and beverages (which are not surface applications but are mixed in) expose intestinal microflora to increased concentrations of natamycin in the gut. According to the authors, this could increase the potential risk for development of polyene resistance in resident *Candida albicans* and *Saccharomyces cerevisiae* within the gut. The level of potential natamycin exposure from beverages presented in the report (500 ppm) far exceeds what is allowed according to the GRAS determination for use in beverages (5 ppm). However, the authors maintain that even at levels currently permitted by regulation which are well below the ADI, the fecal concentration of natamycin may exceed its minimum inhibitory concentration (MIC) (Dalfhoff 2015). The MIC is the lowest concentration of a substance (e.g., natamycin) that inhibits the growth of a target species, such as *Candida sp.* Increased exposure of a target organism to a substance can lead to an increased MIC, which indicates that the target organism's susceptibility to the substance has been diminished. Dalhoff and Levy (2015) based their claim regarding the potential development of natamycin resistance in part on a study which reported on the effects of natamycin administered orally in combination with butylscopolamine for the treatment of intestinal candidosis at a daily dose of 400 mg for 10 days in 356 individuals. Dahloff and Levy claim that the results showed that the susceptibility of *Candida spp.* to natamycin was significantly reduced during the exposure period and that it returned to normal levels when checked 3 months post-exposure. However, as Streekstra, Keuter and Wilms (2015) point out in their response to Dalhoff and Levy (2015), the original authors of the study concluded that there had been no marked changes to the MIC of natamycin as a consequence of the natamycin treatment (Streekstra, Keuter and Wilms 2015) (Gehring, et al. 1990).

In general there is a lack of evidence in the literature to show that applications of natamycin in food at regulatory-approved levels lead to fungal resistance as has been seen in certain medical applications (Kaushik, et al. 2001) and other laboratory studies.

The use of natamycin as an antifungal agent in food may have some benefits to human health, namely, the suppression of mycotoxins that contaminate food. Mycotoxins are secondary metabolites of certain fungi which can be carcinogenic, teratogenic, hemorrhagic, or dermatitic. Several studies have shown natamycin to inhibit the production of mycotoxins and molds that produce them (Delves-Broughton, et al. 2005). For example, Medina et al. (2007) found natamycin to be very effective in controlling the production of ochratoxin A over a range of available water and temperature conditions on grape-based media (Medina, et al. 2007).

Natamycin is one of numerous polyene antifungal agents used in medical applications. It is used topically to treat fungal infections of the eye. Specifically, it acts against fungal keratosis, as well as a broad spectrum of other fungi, yeasts, and some protozoa and algae. It was previously used topically in humans against fungal infections of the skin and mucous membranes applied in the form of a cream, ointment, suspensions or tablets; however, current medical use is confined to topical treatment of fungal infections of the cornea and to prevent such infections in contact lens wearers (WHO 2002).

Natacyn® is the FDA-approved antifungal drug for topical ophthalmic administration with natamycin as the active ingredient. Its label describes the active ingredient as a tetraene polyene antibiotic which has *in vitro* activity against a variety of yeast and filamentous fungi, including *Candida*, *Aspergillus*, *Cephalosporium*, *Fusarium* and *Penicillium*. It describes the mode of action similar to that described by the petitioner for control of fungal diseases in agricultural commodities – through binding of the molecule to the sterol moiety of the fungal cell membrane. The label also states that natamycin is not effective *in vitro* against gram-positive or gram-negative bacteria. Further, systemic absorption is not expected with topical use of the product on the eye and gastrointestinal absorption is very poor (Alcon Laboratories, Inc. 2008). Potential side effects from use of the drug are listed as: allergic reaction, change in vision, chest pain, corneal opacity, dyspnea, eye discomfort, eye edema, eye hyperemia, eye irritation, eye pain, foreign body sensation, paresthesia, and tearing (Alcon Laboratories, Inc. 2008). However, these potential risks are not associated with natamycin in the literature, but may be due to inactive ingredients in Natacyn®. One is a preservative, benzalkonium chloride (BAK), which is a quaternary ammonium that has been shown to have allergenic and toxic effects in various studies (Baudouin, et al. 2010).

The label associated with the petitioned use of natamycin as an agricultural fungicide includes the health warnings: "Harmful if swallowed. Causes moderate eye irritation. Avoid contact with eyes. Wear protective eyewear. Wash thoroughly with soap and water after handling and before eating, drinking, and chewing gum, using tobacco, or using the toilet. Remove and wash contaminated clothing before reuse." However, similar to the ophthalmic drug label, these risks are not clearly linked to natamycin in the literature and may be due to the presence of other undisclosed ingredients.

Evaluation Question #11: Describe all natural (nonsynthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

Controlling fungal diseases affecting mushrooms is theoretically challenging as both host and pathogen are from the same taxonomic kingdom and potentially susceptible to the same materials. Additionally, the potential for consumers to ingest pesticides on mushrooms and post-harvest handled fruit requires that fungicides must have low toxicity to mammals (Gandy and Spencer 1981). NOP regulatory allowances differ for materials used as fungicides in mushroom production and post-harvest handling so these uses are discussed separately below.

Nonsynthetic alternatives for mushroom production

Nonsynthetic substances may be used for disease control, unless prohibited or limited at §205.602. Natamycin may be considered a nonsynthetic substance, based in the information provided in *Evaluation Question #3*. Additional nonsynthetic controls such as thyme oil have demonstrated the ability to reduce the incidence of *Verticillium fungicola* (causal agent of dry bubble disease) both *in vitro* (Tanović, et al. 2009), and in mushroom houses (Beyer 2015). As an active ingredient, thyme oil is exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and may not need to be registered for legal use (EPA 2017c).

Aerated spent mushroom substrate (SMS) tea inhibited 100 percent of *V. fungicola* mycelial growth, compared with prochloraz, which inhibited 91 percent mycelial growth. Cropping studies of SMS formulated with peat showed 34 to 73 percent disease reduction, while prochloraz reduced disease by 4 to 7 percent (Gea, et al. 2014). Furthermore, no negative effect on mushroom growth occurred through the use

of the SMS tea. Gea speculated that production of strong iron-chelating compounds (siderophores) produced by specific bacteria (pseudomonads) may have been involved in suppression of *V. fungicola*.

Mushroom alcohol (1-octen-3-ol) shows encouraging results in reduction dry bubble disease. It is registered with the EPA for use as an insect attractant, but not currently for enclosed mushroom production. The substance is responsible for the odor of mushrooms and produced by *Agaricus bisporus* (button mushrooms) through the enzymatic cleavage of linoleic acid. Berendsen demonstrated that when concentrated, the volatile compound was able to inhibit spore germination of *V. fungicola*. Application of a 1.25 percent solution of 1-octen-3-ol in small and commercial scale studies was as effective as prochloraz-manganese in reducing dry bubble disease. 1-octen-3-ol affected is not selective though, and mushroom yield was also reduced somewhat (Berendsen 2011).

Synthetic alternatives for mushroom production

Synthetic fungicides allowed for use in organic crop production include materials at §205.601(i): aqueous potassium silicate (derived from naturally occurring sand), fixed coppers, copper sulfate, hydrated lime, hydrogen peroxide, lime sulfur or elemental sulfur, horticultural and narrow range oils, and potassium bicarbonate. Many of these are not well suited for use in enclosed mushroom production, due to toxicity or insufficient selectivity. Cropping studies conducted by Pennsylvania State University found that paraffin oil (which may be allowed under the NOP definition of narrow range oil) was similarly effective as natamycin in controlling *Verticillium fungicola*; they both showed some control over *V. fungicola*, but control was reduced during the second flush of mushroom growth (Beyer 2015).

Nonsynthetic alternatives for post-harvest handling

Nonsynthetic substances may be used on raw agricultural commodities post-harvest, unless prohibited or limited at §205.602. Examples of materials that could theoretically be used to prevent spoilage include: nitrogen gas, nonsynthetic microbial preparations, glucosinolates (from plants in the family Brassicaceae) and vaporized acetic acid. Vaporized acetic acid acts as a disinfectant and is applied directly (Sholberg and Gaunce 1995). When tested on a wide variety of fruits, Sholberg found that low concentrations (≤5.4mg/L) of vaporized acetic acid significantly reduced post-harvest decay caused by *Penicillium expansum* and *Botrytis cinerea,* and the treatment itself did not cause additional fruit damage. No information on commercial products utilizing the technology was found.

Microbial preparations such as Bio-Save® 10LP Biological Fungicide (JET Harvest Solutions; Apopka, FL) based on *Pseudomonas syringae*, act as antagonists to decay causing fungi. Mechanisms of action include competition for nutrients and space, production of anti-fungal metabolites, parasitism, and reducing pathogen enzyme activity (Mari, Bertolini and Pratella 2003). Apples wounded and inoculated with blue mold (*Penicillium expansum*) were left untreated or treated with *Pseudomonas syringae* (Bio-Save 10LP), cyprodinil, thiabendazole, or a combination. At a concentration of 2.8 X 10^8 CFU/ml, the *P. syringae* treatment reduced blue mold 100 percent (Errampalli and Brubacher 2006). Field trials using another *P. syringae* product (Bio-Save 100) showed a significant reduction in disease incidence of wounded apples after two weeks of storage at 13°C as compared with a water control (Chen, et al. 1997).

Coatings such as waxes and shellacs, listed at §205.605(a) and §205.606, respectively, are processing materials that can decrease plant tissue senescence (ripening), and thus help delay the point at which spoilage due to fungi occurs (Lin and Zhao 2007).

At least one organism that produces natamycin, *Streptomyces lydicus* is registered with the EPA as an active ingredient for use in pesticide products and is used in 21 registered products (EPA 2017b). There are 6 products on the OMRI List as of July 2017[12] that declare *S. lydicus* on the label (OMRI 2017b).

[12] Two of these six OMRI Listed products are not EPA Registered because they are not intended for sale in the United States, and therefore are not subject to EPA regulation.

Synthetic alternatives for post-harvest handling

NOP Guidance 5023: Substances Used in Post-Harvest Handling of Organic Products clarifies that synthetic crop input materials listed at §205.601 are not permitted for post-harvest use, unless specifically annotated as such; there are no substances on §205.601 permitted for the petitioned post-harvest uses. Therefore, synthetic alternatives for post-harvest fungicidal applications are limited to those found at §205.605(b). Decay causing fungi are spread to fruit and harvest bins in the field, and subsequently spores are transferred in processing waters (Mari, Bertolini and Pratella 2003). Materials that could be used to prevent or slow decay include acidified sodium chlorite, hydrogen peroxide, ozone, peracetic acid, and chlorine materials, in accordance with any annotations or restrictions. Many products exist that contain these materials which disinfect the surface of produce as well as processing water (OMRI 2017b).

Carbon dioxide and nitrogen can be used in controlled atmosphere storage which slows ripening, delaying fruit softening and subsequent spoilage, and is a commonly used technology (Bapat, et al. 2010) (Thompson 2016).

Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

Mushroom production alternative practices

Pathogenic fungi such as *Trichoderma* and *Verticillium* species can exist in mushroom growth substrates (e.g., compost, casing). *Verticillium fungicola*, the causal agent for dry bubble disease is abundant in materials that are used for casing, and is spread on infected equipment, hands, clothing, water, dust, and by vectors such as mites and insects (Sharma, Kumar and Sharma 2007) (Gea, et al. 2014). Beyer reported that a single infected mushroom could produce 30 million spores in an hour (Beyer n.d.), and spores can survive in moist soil for one year (Sharma, Kumar and Sharma 2007). Vegetative mycelium of *Agaricus bisporus* (button mushroom) is resistant to infection, but sporocarp (mushroom) related tissue is highly susceptible (Berendsen 2011). Sporocarp tissue develops in the mushroom casing, and so hygiene for this part of the growth substrate is especially important. Fully resistant cultivars are not known, though some strains have shown partial resistance (Berendsen 2011). Symptoms include deformed sporocarp tissue, splits in the stem, and necrotic spots or blotches (Beyer n.d.).

Disease prevention strategies largely revolve around hygiene. Farms, equipment, and personnel must be kept clean. Casings can be heat or steam treated, which has been demonstrated to prevent spore germination (Sharma, Kumar and Sharma 2007). The condition of the underlying compost is less critical to disease development, with only very high spore concentrations able to induce disease (Beyer n.d.). Controlling dust and limiting water movement within the house is necessary to prevent moving an infection from one area to another. Water splashed while cleaning floors can cause disease epidemics, so low-pressure, or waterless floor cleaning methods are preferable. Controlling vectors such as flies and mites before they can spread spores is necessary (Gea, et al. 2014). In vitro studies indicate that reduced susceptibility can also be achieved through the use of strains that form fruiting bodies earlier (Berendsen 2011). Infected mushrooms should not be disturbed or removed, but can be covered in salt or alcohol (Beyer n.d.).

Post-harvest disease management

Post-harvest disease management strategies are crop-specific and well described in literature. Generally speaking, hygiene is important to the prevention of disease (Suslow 2000). Diseased or wounded fruit should not be intermingled with fruit in good condition. Fruit should be cooled as quickly as possible. Storage life for fruits (and prevention of decay) varies depending on cultivar, climate, harvest timing, and nutritional conditions. Common fungi that cause decay in post-harvest fruits include *Botrytis cinerea* (gray mold), *Colletotrichum acutatum* (anthracnose), *Mucor piriformis* (mucor rot), *Penicillium spp.* (green mold, blue mold), and many others (Smilanick 2011) (Mari, Bertolini and Pratella 2003) (Almenar, et al. 2007). As fruit ages it undergoes physiological changes during ripening and senescence such as increased respiration rate, ethylene production, conversion of starches into sugars, and softening due to changes in cell walls (Thompson 2016). These processes can increase susceptibility of produce to fungi. After disinfection (if

possible), refrigeration and controlled atmosphere storage can be used to control these physiological processes and prevent or delay the fruit's susceptibility, or slow infections.

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