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.

Petition to Add Kasugamycin to the National List

Item A.1 — Indicate which section or sections the petitioned substance will be included on and/or removed from the National List.

- Synthetic substances allowed for use in organic crop production (§205.601)

Item A.2 — OFPA Category - Crop and Livestock Materials
• Toxins derived from Bacteria

Item B—

1. Substance Name

Common Name: Kasugamycin.
Alternate formulated names: Kasugamycin monohydrochloride, Kasugamycin hydrochloride hydrate
Generic Names: Kasugamycin: 3-O-[2-amino-4-[(carboxyiminomethyl) amino]-2,3,4,6 tetradeoxy-"-D-arabino-hexopyranosyl]-D-chiro-inositol
Kasugamycin monohydrochloride: D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-alpha-D-arabino-hexopyranosyl)-, monohydrochloride

2. Petitioner and Manufacturer Information

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3. Intended or Current Use

For use to control Fire Blight disease, caused by the bacteria *Erwinia amylovora* in apples, pears and quince (pome fruit). We suggest an annotation to limit it to this use, as there are a few other diseases of other tree crops listed on the label. A fact sheet from Washington State University with an overview of the disease and control measures is included as Appendix A.

4. Intended Activities and Application Rate

Sprayed on trees at a rate of 5 liters/hectare (64 fl. oz./acre in 100 gallons of water), starting at 20 to 30% bloom or when conditions favor disease development. Continuing at 7-day intervals when conditions favor disease development, but not to exceed more than 4 applications or 20

liters per hectare per year.

Label states to not apply within 90 days of harvest or after petal fall. Label for currently formulated product (not organic) is appendix B along with general information about kasugamycin from the manufacturer.

5. Manufacturing Process

(Note: This information has been provided by the manufacturer.)

The process for the production of kasugamycin comprises cultivating a strain of *Streptomyces kasugaensis* in an aqueous carbohydrate solution containing a nitrogenous nutrient under submerged aerobic conditions until a substantial quantity of kasugamycin is formed.

Kasugamycin is a naturally occurring compound that is isolated from the soil bacterium *Streptomyces kasugaensis.* It is produced on a large scale by aerobic fermentation of *Streptomyces kasugaensis* followed by several purification steps. First step in the process is generating *Streptomyces kasugaensis* in a seed-culture fermentation and then expanding the seed culture into a main fermentation for commercial production. Fermentation media are prepared *in situ* and sterilized before each fermentation step.

At the end of fermentation, several steps are taken to sterilize and remove non-ions and sodium ions by ion exchange, followed by evaporation to concentrate the product before spray drying and packaging the product. By carefully monitoring the temperature during the entire process, the quality and optimum growth of the microorganism is guaranteed. Due to the fermentation nutrients, possible impurities include water, peptides, hexoses/pentoses, and amino acids.

6. Ancillary Substances

N/A

7. Previous Reviews

This substance has not been reviewed for organic production by any state or federal bodies.

8. Regulatory Authority

Kasugamycin is approved and registered by the EPA. The registration number of Kasumin 2L (the current formulated product which does not have inerts that meet organic regulations) is 30591. The tolerance was established in 40 CFR §180.614 for Vegetable, Fruiting Group 8 at 0.04 ppm. The 2005 EPA fact sheet is an appendix.

Canada has registered Kasugamycin. Their summary is at this link (not downloadable): https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/consultations/kasugamycin-proposed-registration-decision-prd2012-30-health-canada-consultation-document.html

Japan conducted a risk assessment in 2014, with an abstract in English at this link: https://www.fsc.go.jp/english/evaluationreports/pesticide/kasugamycin_fs246.pdf

9. Chemical Abstracts Service (CAS) Number and Product Labels

The CAS number is 6980-18-3. An alternate CAS number for kasugamycin monohydrochloride is 19408-46-9.

Product label and information are attached in Appendix B. Note that the current product, Kasumin 2L, is not allowed for organic production even if Kasugamycin is approved because the inerts are not compliant. However the company has told the petitioner that they are interested in formulating an organic version if the active ingredient is approved.

10. Physical and Chemical Properties

Provide the substance's physical properties and chemical mode of action including the following:

TABLE 2. Physicochemical Properties of the Technical Grade Compound (Kasugamycin Hydrochloride Hydrate).

Parameter	Value	Reference
Molecular Weight	433.8	MRIDs # 45910004 and -05
Melting Point/Range	202-230°C (decomposing)	
pH	4.35 at 24.5°C (1% wt/vol solution)	
Density	0.43 g/mL at 24.5°C	
Water Solubility	g/100 mL pH 5 20.7pH 7 22.8pH 9 43.8	
Solvent Solubility	g/100 mL Methanol 0.744 Hexane $<1 \times 10^{-5}$ Acetonitrile $<1 \times 10^{-5}$ Methylene chloride $<1 \times 10^{-5}$	
Vapor Pressure	<0.013 mPa at 25°C	
Dissociation Constant (pK_a)	$pK_{a1} = 3.23$ $pK_{a2} = 7.73$ $pK_{a3} = 11.0$	
Octanol/Water Partition Coefficient (Log [K_{OW}])	<1.96 at 23°C and pH 5	
UV/Visible Absorption Spectrum	Not available	

--Table copied from EPA fact sheet in Appendix C.

Other relevant characteristics of kasugamyin are that it has zero residue at harvest, rapid degradation in soil, and is never applied within 90 days of harvest.

(a) Chemical interactions with other substances, especially substances used in organic production;

Since Kasugamycin will have an adverse effect on gram-negative bacteria, it may interact with such bacteria in the soil and on plants. This includes some biological organisms that may also be applied for disease control. Therefore it is recommended to spread the applications apart by several days. However no specific interactions have been studied.

(b) Toxicity and environmental persistence:

In a study of the degradation of kasugamycin in water, it was found that while it is generally nontoxic to microorganisms, it can inhibit the growth of some aquatic bacteria but also stimulate the growth of other bacteria. The conclusion was that kasugamycin is not a persistent pesticide in water. (Huang 2010). Another experiment in rice paddies to evaluate residue in water showed that the risk of water contamination was very low. (Sheu, 2010).

(c) Environmental impacts from its use and/or manufacture:

Unlike the antibiotic Streptomycin which now has high levels of resistance in the *Erwinia amylovora* population, there have been no bacterial colonies found in the phyllosphere with resistance to kasugamycin (Tancos, 2017). While there was one report of resistant soil bacteria when cultured on media containing high concentrations of the antibiotic (McGhee & Sundin, 2011), there is no indication that this would have an effect in the orchard soil outside or within the tree canopy.

(d) Effects on human health:

"Kasugamycin exhibits low acute toxicity, being only a mild dermal and ocular irritant. The major effects observed across species in multiple-dose studies were decreased body weights and body weight gains." (Federal register 2014, attached Appendix D)

"Although antimicrobial drug residues present in or on food may cause adverse effects on the ecology of the intestinal microflora of consumers, the Agency does not believe this is a concern for kasugamycin because of the use pattern (application occurring prior to fruit development) and low residue detection in field trials." (Federal register 2014)

"Based on these risk assessments, EPA concludes that there is a reasonable certainty that no harm will result to the general population or to infants and children from aggregate exposure to kasugamycin residues." (Federal register 2014)

“Because kasugamycin is active only against phytopathogenic fungi and bacteria, it has never been employed as a human or veterinary-use antibiotic.” (EPA Fact Sheet 2005, Appendix C)

An early study on the antibacterial activity of kasugamycin supported that it did not have any appreciable effect against most bacteria that affect humans. The conclusion was, "Further evaluation of kasugamycin for potential human use as an antipseudomonal agent does not appear warranted." (Levitan, 1967)

(e) Effects on soil organisms, crops, or livestock.

No studies could be found that specifically looked at soil organisms or crops, other than the studies that show rapid degradation of the substance in the environment, which therefore can be presumed to not have an effect on soil or crop.

One study did look at non-target bacteria inhabiting apple flowers and leaves. (McGhee & Sundin, 2011). It found that spraying kasugamycin did result in smaller post-application bacterial populations than either water or tetracycline sprays in flowers. They also found that many nontarget bacteria in apple orchards are not sensitive to kasugamycin.

11. Safety Information

An MSDS for Kasugamycin is attached as Appendix E.

12. Research Information

The research papers referenced in this petition can be found in Appendix F. Those marked with an asterisk (*) are attached as other appendices.

13. Petition Justification Statement

Provide a "Petition Justification Statement," which provides justification for any of the following actions requested in the petition:

A. Inclusion of a Synthetic on the National List (7 C.F.R. §§ 205.601, 205.603, 205.605(b))

A1. Explain why the synthetic substance is necessary for the production or handling of an organic product.

The removal of the antibiotics Streptomycin and Oxytetracycline from the National List in 2014 has had significant effects on the production of organic apples and pears nationwide. The three primary issues are continuing large crop and tree losses to fire blight, the fact and perception that the alternative controls available do not work in many specific situations, and a competitive disadvantage becoming more acute between Washington state and other apple-producing regions. These three issues are described in more detail below.

Kasugamycin is an antibiotic, but one that has no use in human or animal medicine. This makes the primary argument of those who opposed Streptomycin and Oxytetracycline irrelevant, since there is no evidence to suggest that resistance to other antibiotics can transfer to humans from this one. In this sense it is similar to the recently approved material Polyoxin D zinc salt. Polyoxin D is similar in the way it is produced and is effective against fungus, but has no role in human or veterinary medicine.

What is most important to consider is that kasugamycin works better than most other materials to control fire blight. It even works better than the other antibiotics. (Aćimović, 2017), (Adaskaveg 2016, 2017, 2018, 2019) (DuPont, 2019), (Tancos, 2017). It also has so far shown less resistance potential than streptomycin and is effective in a wide range of situations.

Losses from Fire Blight

Organic growers have experienced heavy losses from fire blight since 2015, the first growing season without antibiotics. The experiences are mainly anecdotal, because there was no quantification of losses to be found in the literature. The growers highlighted below are from the West, because that is where the petitioner is located. Other regions will undoubtedly offer their own experiences once the petition is available to the public for comment.

A couple of photos are included in Appendix G.

Grower 1 is a large organic apple grower in the Central Valley of California. He completely lost a 10-acre block of Pink Lady to fire blight. He is not planning to plant apples in that block again. The disease travelled into his adjacent 40-acre block of Galas, where it has caused a major loss of revenue due to loss of tree structure from pruning out infection and thus loss of bearing capacity. It has also resulted in a large extra cost of production for removing infected wood, and using a large quantity of high-cost alternative treatments of limited effectiveness. The climate in his region is very warm early in spring and wet from fog and late rains. These conditions are highly conducive to fire blight.

Grower 2 is a small organic farm in Sonoma County, California. On 14 acres they grew 9 acres of Asian Pears, 2 acres of apples, and a variety of other fruits. They lost about one-third of their productive fruiting wood, especially on the Asian pears, which are highly susceptible to fire blight. They spent the intervening years since 2015 trying all the alternative materials and are now using a hydrogen peroxide material for some control, but have had severe economic losses and have not fully recovered.

Grower 3 is also from the Central Valley and he considers himself small with 150 acres of fruit. In 2018 and 2019 they lost 17 acres of third-leaf apples and 16 acres of third-leaf Asian pears to fire blight. On their more established orchards they lost 85% of the flower clusters in 40 acres of established apples, which devastated two years of production while the trees grew back and flowered. This loss was more than $1,600,000. While other farmers in the area removed apple orchards and replaced them with conventional fruit, Grower 3 decided to keep looking for an organic solution. The only thing that kept them afloat is their diversification in other types of fruit. This farm began doing trials of alternative controls in 2012, two years before the loss of antibiotics. However they have not had much success until recently when they have their fingers crossed about a new regimen. However 2020 is not shaping up to be a year with high fire-blight pressure due to the cool spring).

Grower 4 is from Washington state. They lost an 8-acre block of pears to fire blight in 2017 as well as much of another 20-acre block. They are on a 5th-generation homestead and cannot afford to replant the 8 acres. They took the rest of their orchard out of organic and are using conventional methods to try and salvage their other trees.

These four growers are a drop in the bucket among the organic orchardists around the country. When this petition comes before the NOSB, many more growers will share their experiences.

Alternative Controls Do Not Work

Dr. Granatstein did an unofficial survey in 2015 of growers in Washington, California, and

Wisconsin about their success with controlling fire blight without antibiotics. Over 70% of Washington growers said they were successful, less than 30% did in California, and about 50% in Wisconsin. (Granatstein, personal communication). He did find that perception of fire blight as a problem varied between years. This is to be expected, as fire blight is worse or better in some years in all regions.

The reasons the alternatives do not work is almost as varied as there are growers. Here are some of the main reasons:

- Lack of Models for All Regions
  Models are how growers (in regions that have models) decide on the timing of their spraying practices. Models are only as good as the information that is given to them and so a model based on weather in Washington will not accurately predict when to spray in California.
- Timing of Sprays
  Growers in some regions are trying to manage apple scab, plum curculio, leaf rollers, aphids, and other pests at the same time as fire blight. This means they have to juggle the spraying of oils, copper, and lime-sulfur with biological products which are not compatible with those other products. Not only that, but it all must be timed around rain and warm weather, which can lead to spraying too late, missing an application, or other problems.
- Resistance of *E. amylovora* to Copper
  Recent research from the California Apple Commission is indicating that the organism that causes fire blight is starting to become resistant to copper (Adaskaveg, 2018, 2019, attached as Appendix G). Surveys of copper resistance in California pear-growing areas showed moderate copper resistance. Management failures with use of copper under high disease pressure have been attributed partly to this resistance. Pears are highly dependent on the use of copper since lime sulfur and Blossom Protect cause extreme russeting of pears.
- Lack of a Suitable Choice of Product for Late (Rat-tail) Bloom and Post Bloom
  Almost all the products on the chart presented below are only registered or effective when sprayed during bloom. The warmer areas of the country, such as California, often get less chill hours in the winter which causes bloom to be spread out over a 6-week period rather than the normal 3 weeks experienced in cold climates. Since the end of that 6-week period is quite warm and can be very moist, ideal conditions may exist to encourage fire blight. Spraying many products at that time is not feasible because it will kill or cause russet on fruit which has already set. In other parts of the country, summer hail storms create extensive wounds on leaves after bloom is finished, and the fire blight enters through these wounds. There are few, if any, products that can be sprayed at this time.
- Genetic Elasticity of *E. amylovora*
  It is apparent that the bacteria causing fire blight can mutate very readily. It can become so specific to a certain microclimate that it is virulent at one location, but not nearly as bad at another orchard a mile away. Factors such as wind direction and speed, relative temperature between night and day, humidity build-up, topography, and other factors are so microclimate-specific that research cannot account for every situation.

Some instances of products not working are also undoubtedly due to lack of training for growers on how to use certain products, on competition between products that retard effectiveness, and on errors in correct and timely applications. However, there are enough significant reports from

orchard managers who do everything right and still get substantial disease, that the need for a more reliable and safe product such as kasugamycin is extremely important.

Competitive Disadvantage Between States

Apples ranked second in the top 10 Organic Fresh Produce Items in 2018 (Granatstein and Kirby, 2019). According to the 2015 NASS Organic Production Survey (referenced from Granatstein above), Washington State produces 71% of the reported organic apple acres with 93% of the fresh fruit volume, and 57% of the organic pear acres with 79% of the fresh fruit volume.

Organic Apple Area (acres, estimated)

State	2003	2005	2007	2008	2011	2014	2015
WA*	7,003	6,721	8,018	12,936	14,296	14,052	14,283
CA*	4,045	3,402	3,900	3,393	2,322	3,392	3460
AZ	835	865	816	816	354	?	?
CO	235	202	209	164	509	194	176
OR	265	123	106	136	234	262	143
Other West	171	83	147	139	96	17	59
West total	12,554	11,396	13,196	17,584	17,934	17,917	18,121
Midwest	650	708	612	655	1,207	319	563
NY & NE	5	392	212	193	361	645	555
S & SE	1	8	47	33	40	11	10
US Total	13,210	12,504	14,067	18,465	19,542	19,370	20,156
*WA and CA values are from WSDA, OTCO, CCOF, and CDFA							

Table from Granatstein & Kirby, 2019.

The above table makes clear that Washington state keeps increasing their apple volume while many other states are going down or at best stabilizing. Limited information from Granatstein 2019 and the popular press indicates that the disparity is getting bigger each year since 2015.

In 2016 there was a very severe fire blight epidemic in New York state. One of the papers that looked at alternatives in general contained the following statement:

> "Based on studies associating global warming with changes in plant pest ranges and degree of infestations, we predict that years with very favorable weather for fire blight epidemics will become more frequent in cool climate regions of the US and the rest of the world." (Aćimović, 2018)

In Michigan there was a bad fire blight epidemic in 2000 which wiped out over 400,000 apple trees and caused an estimated $42 million in damages (Michigan State, 2013). In California one of the worst years was 2015 where the very dry warm winter resulted in heavy losses and infected apples on the coast as well as the Central Valley. Many coastal zones normally only get fire blight on pears.

A2. Describe any nonsynthetic substances, synthetic substances on the National List, or alternative cultural methods that could be used in place of the petitioned synthetic substance.

Control of fire blight is very complicated because the disease expresses itself differently in different regions, in different years and on different fruit varieties. When the antibiotics Streptomycin and Oxytetracycline were removed from the National List with only slightly over a year of notice, the research being done on alternatives was quite rudimentary and was mostly being done in the Pacific Northwest. What has shown to work well for Washington and Oregon on certain varieties, has not worked at all well in California, New York, Michigan, and other apple-growing states. Even in the Northwest, the protocol developed has not performed as well on some varieties such as Pink Lady or Gala, and in all seasons with changing weather patterns.

A thorough discussion of organic fire blight control is given in the report from the Organic Center in 2013 (Ostenson and Granatstein, 2013). None of the methods or materials discussed in this report or here in this petition is effective by itself, but some or all must be used in an integrated context to achieve sufficient control in some years and inadequate control in others.

Cultural methods that help with control include cultivar and rootstock selection; using models to predict conditions susceptible to disease development; pruning and training for better air circulation; crop load management; orchard sanitation; and use of pre-bloom foliar nutrients. Some of these are more feasible than others and some have considerable limitations. For instance models for the Pacific Northwest or the Northeast do not do well in California because they do not account for the warm foggy nights in the Central Valley that are ideal for disease development and spread. Varieties are generally driven by the marketplace and it is not easy to just switch varieties, even if a truly resistant variety were developed (and not many have been developed so far).

The table below contains organic materials that have been used in organic fire blight control. Some are the well studied ones that were used prior to the 2014 sunset of antibiotics, and some are new since then and do not have a lot of published research to back them up. Although this looks like a long enough list that something should work for everyone, that has not proven to be the case. Many of the materials cannot be used at the same time, or do not work in some situations, or have very narrow timing windows that may make them ineffective, and have not been objectively studied in reputable studies. Because the consequence of failure is losing all the trees in an orchard, growers need assurance that a material will work before risking their whole livelihood on it.

Organic Products for Fire Blight (per label claim)

Product	Supplier	Active Ingredient	Mode of Action
Brandt Organics Aleo	Brandt Consolidated	garlic oil	contact kill
Blossom Protect	Westbridge Agricultural	*Aureobasidium pullulans* strains DSM14940 & 14941	competitive with pathogen
Bloomtime Biological	Northwest Agricultural	*Pantoea agglomerans* strain E325	competitive with pathogen
BlightBan	NuFarm	*Pseudomonas fluorescens* strain A506	competitive with pathogen
Serenade Optimum	Bayer	*Bacillus amyloliquefaciens* strain QST713	antibiotic metabolites
Double Nickel	Certis USA	*Bacillus amyloliquefaciens* strain D747	antibiotic metabolites
Serifel	BASF	*Bacillus amyloliquefaciens* strain MBI600	antibiotic metabolites
Regalia	Marrone Bio	extract of *Reynoutria* (giant knotweed)	resistance inducer
LifeGard	Certis USA	*Bacillus mycoides* isolate J	resistance inducer
Lime Sulfur	*(several companies)*	Calcium polysulfide	contact kill
Copper	*(several companies)*	Copper (hydroxide, octanoate, or pentahydrate)	contact kill
Prestop WG	Lallemand Plant Care	*Gliocladium catenulatum* strain J1446	competitive with pathogen
Agriphage-Fireblight	Certis (Omnilytics)	Bacteriophage active against *Erwinia amylovora*	*(see reference to Fruit Grower News 2019)*
Jet-Ag	Jet Harvest Solutions	Peracetic Acid*	contact kill

* many other brands available
Table created from Wallis et al. (2019), Zipp (2019), Courtney (2019), Aćimović (2017), and manufacturers' websites.

Since 2014 researchers in a few states (Michigan, New York, California) have started to study some of the alternative materials. (Wallis et al. 2019), (Zipp 2019), (Courtney 2019), (Aćimović 2017), (Elkins 2015) (Adaskaveg 2016, 2017, 2018, 2019). Almost all of these articles state that none of the treatments work as well as the antibiotics, and many of them use both streptomycin and kasugamycin treatments as comparison as well as untreated controls.

"Even though eco-friendly and/or organically acceptable options for management of fire blight are much more available today than before, their efficacy is still not as reliable as the efficacy of streptomycin and kasugamycin." (Aćimović 2014, quoting Sundin 2014)

Pears are of particular concern in the inadequacy of organic control measures. This is partly

because many pear varieties are prone to russeting when copper or lime sulfur is used, or timed wrong. Even Blossom Protect has led to russeting problems in pears and some apples. Popular pear varieties are extremely susceptible to fire blight, especially Asian pears, which are widely grown in the Central Valley of California. But now organic ones are very scarce because they are so susceptible and none of the above control measures have worked. The few pear varieties that are promoted as resistant to the disease are either not completely resistant in all seasons or locations, or else have major marketing drawbacks such as lumpy shapes, rough skin, russeting, or small size that prevent them from gaining traction in most markets.

In the course of the NOSB's review of this petition, much public comment will be provided from growers and scientists in various regions about alternatives they have tried, why they didn't work satisfactorily, and what losses they have experienced. Because the disease is so prevalent and yet so specific in each microclimate, these personal experiences will paint a more thorough picture than can be fully described in this petition. Suffice it to say that the existing organic options are not controlling the disease well in many regions and another safe organic option such as kasugamycin would be welcomed.

A3. Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a nonsynthetic substance or alternative cultural method.

The primary beneficial effect to the farm ecosystem is that the orchard will not die from fire blight if this substance can be used. The overall ecological footprint of kasugamycin is substantially less than that of copper, and even less than that of lime sulfur. Approval of kasugamycin could have a very positive effect on human health as well, because it could enable more orchards to transition to organic production in parts of the country outside of the Pacific Northwest, and this would result in far less toxic conventional pesticides being used as well as less streptomycin and tetracycline.

Conclusion

Research is ongoing in some states for products that have effectiveness in control of fire blight disease. Almost all the research indicates that kasugamycin works consistently in all the states that have studied it, while other materials show much less consistent results across years, regions, varieties, and local conditions.

The research results are not coming out fast enough for growers who are in high disease-pressure regions. These growers have already had huge losses to fire blight and in some cases have stopped growing apples and pears in favor of other crops that are less complicated such as grapes or nuts. Growers who wish to keep their orchards must remain diligent at practicing all the cultural and biological controls they can, and must do everything possible to stay ahead of this devastating disease.

The California Apple Commission urges the NOSB to approve the safe and effective material Kasugamycin for use in organic production of pear, apple and quince. It meets all the criteria in the Organic Foods Production Act for a material to be added to the National List.

List of Appendices for Kasugamycin

Appendix A
Washington State University Fire Blight Fact Sheet 2019

Appendix B
Kasumin Label and Background Information from UPL.

Appendix C
EPA Fact Sheet on Kasugamycin 2005.

Appendix D
Kasugamycin; Pesticide Tolerances.
Federal Register Notice Vol. 79, No. 186 2014, pp. 51492 - 51497

Appendix E
Kasugamycin Materials Safety Data Sheet

Appendix F
References and Research Information

Appendix G
California Apple Commission Annual Report 2018-19 (Adaskaveg)

Appendix H
Photos of Fire Blight Infected Organic Orchards

Fire Blight of Apple and Pear

Appendix A

Updated by Tianna DuPont, WSU Extension, February 2019. Original publication by Tim Smith, Washington State University Tree Fruit Extension Specialist Emeritus; David Granatstein, Washington State University; Ken Johnson, Professor of Plant Pathology Oregon State University.

Overview

Fire blight is an important disease affecting pear and apple. Infections commonly occur during bloom or on late blooms during the three weeks following petal fall. Increased acreage of highly susceptible apple varieties on highly susceptible rootstocks has increased the danger that infected blocks will suffer significant damage. In Washington there have been minor outbreaks annually since 1991 and serious damage in about 5-10 percent of orchards in 1993, 1997, 1998, 2005, 2009, 2012, 2015, 2016, 2017 and 2018.

Casual Organism

Fire blight is caused by *Erwinia amylovora*, a gram-negative, rod-shaped bacterium. The bacterium grows by splitting its cells and this rate of division is regulated by temperature. Cell division is minimal below 50° F, and relatively slow at air temperatures between 50° to 70° F. At air temperatures above 70° F, the rate of cell division increases rapidly and is fastest at 80° F. Above 95°F cell density on and in the plant can actually decline (Pusey and Curry 2004).

Host Range

Considered a problem for apple and pear, *Erwinia amylovora* has a wide host range within Rosacea and Rubus with reports on about 200 species including crab apple, hawthorn, mountain ash and Bradford pear (Timur Momol and Aldwincklke 2000).

Signs and Symptoms

Overwintering cankers can appear black, grey or violet. Older cankers may have dry sunken tissue. If the bark is cut from the edge of an active canker, reddish flecking can be seen in the wood near the canker margin. (Teviotdale 2011).

Figure 1 Canker on apple. Photo T. DuPont, WSU.

Blossom symptoms become apparent one to two weeks after infection. The floral receptacle, ovary, and peduncles become water soaked and dull, grayish green in appearance. Later tissues shrivel and turn brown to black. During periods of high humidity, small droplets of bacterial ooze form on water-soaked and discolored tissues. Ooze droplets start creamy white, becoming amber tinted as they age (Johnson 2000).

Figure 2 Bloom symptoms 12 days after infection. Photo T. DuPont, WSU.

Shoot symptoms. Tips of shoots may wilt rapidly to form a "shepherd's crook." Leaves on diseased shoots often show blackening along the midrib and veins before becoming fully necrotic, and cling firmly to the host after death (a key diagnostic feature.) Numerous diseased shoots give a tree a burnt, blighted appearance, hence the disease name.

Figure 3 Characteristic shepherds crook. Notice ooze. Photo T. DuPont, WSU.

Rootstocks infections usually develop near the graft union as a result of internal movement of the pathogen through the tree or from infection of root suckers. The bark of infected rootstocks may show water-soaking, purplish to black discoloration, cracking, or signs of bacterial ooze. Red-brown streaking may be apparent in cambium just under the bark. Symptoms of rootstock blight can be confused with Phytophthora collar rot. Malling 26 and 9 rootstocks are highly susceptible to fire blight (Johnson 2000).

Figure 4 Rootstock infections may appear water soaked under the bark.

Transmission and Disease Cycle

Erwinia amylovora overwinters within diseased plant tissue (e.g. cankers). In 20 to 50% of cankers active cells survive the winter (van der Zwet and Beer 1991) and when humidity is high in the spring the pathogen oozes out of these cankers. This ooze is attractive to bees, flies and other insects who transfer the blight pathogen to flowers. Pathogen cells can also be moved from old cankers to flowers by splashed and wind-blown rain. Pathogen cells multiply quickly on nutrient rich floral stigmas when temperatures are warm (70-80 F is optimal for the pathogen) (Ogawa and English 1991). Bacterial colonies can then be washed down the style into the floral cup by water (usually from rain or heavy dew) where they can invade flowers through the nectaries. Once initial blossoms are infested, insects and rain can move the pathogen to additional flowers (Pattemore et al. 2014, Johnson et al. 1993). If the pathogen is successful in infecting the developing fruit-let, the disease spreads into the cambium (just between the bark and the wood) of the tree, killing young host tissues as it progresses (Momol et al. 1998) creating characteristic strikes and cankers. Pathogen cells migrate inside the tree well ahead of visible symptoms where they can accumulate in other susceptible tissue such as one-year old shoot tips and susceptible rootstocks causing infections distant from the original infection point. *Erwinia amylovora* can also infect susceptible one and two-year-old tissue directly through wounds (e.g. insect feeding and hail) causing shoot blight infections.

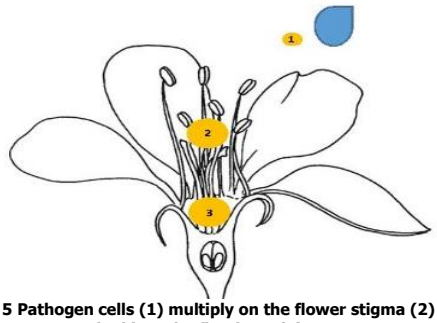

Figure 5 Pathogen cells (1) multiply on the flower stigma (2) and if rain or dew occur are washed into the floral cup (3).

Cultural Controls

Plant on resistant rootstock. Resistant rootstocks (e.g. Geneva series for apples) do not make the scion less susceptible, but will help prevent tree death from rootstock blight.

Sanitation. In winter, cut out old blight cankers as thoroughly as possible. Ideally, cut blight before you prune for tree structure so that the blighted cuttings can be removed from the orchard. Compared to cuts made in summer, winter removal cuts can be made closer to the visible canker edge. In winter the pathogen is confined to the cankered area. Cut at the next "horticulturally sensible" site below the canker. You do not need to sterilize tools when you are cutting on fully dormant trees. Late dormant copper applications may also provide orchard sanitation, reducing inocula levels going into spring (Elkins et al. 2015). During the summer, cut out blight when you see it. Make summer cuts AT LEAST 12-18" below the edge of the visible canker. Cut more aggressively in young, vigorous trees or susceptible varieties. Removing a strike can greatly reduce further damage to the tree if cut early.

Manage the orchard environment. In addition to warm temperatures moisture is required to create infections. As little as two to three hours of wetting is sufficient to trigger infections. Manage weeds/cover crops to limit relative humidity. Do not irrigate during bloom.

Blossom removal in young blocks. Blossom removal in young blocks and removal of late blooms limits the numbers of flowers and thus reduces potential points of infection.

Keep vigor of the tree moderate. Moderating vigor will not prevent infection, but it can reduce damage to the tree.

Temperature Risk Models

The risk of fire blight infections during bloom can be calculated based on the temperature and moisture. In Washington the best prediction model is CougarBlight available at WSU Decision Aid System for Tree Fruit (DAS). This model calculates fire blight risk based on the temperature of the previous four days using the documented growth rate of the bacteria, e.g. higher risk with multiple hours above 70 F. (Pusey and Curry 2004). The model then projects risk for the next three days based on predicted temperatures. Growers can use model information to decide when to spray. If trees are likely to be blooming during an upcoming high-risk period, protective sprays are recommended (Smith and Pusey 2010).

Chemical Control Programs

There is a risk of fire blight infection any time there are flowers on the tree, the weather is warm, and wetting occurs. Watch for and protect secondary blossoms during the three weeks after petal fall, which is the most common time of fire blight infection. Most sprays only protect the blooms that are open. Protect new blooms as they open. In warm weather follow-up sprays are needed every few days.

Conventional Management

Prebloom. Fixed-copper sanitation, but only if fire blight was in the orchard last year.

Early bloom. Apply biologicals (Blossom Protect) during early bloom. If fire blight was in the orchard last year, apply two applications of the biological. Re-apply biologicals a second time if lime sulfur was applied. Lime sulfur applied during early bloom is also antimicrobial and reduces blight pressure.

Early bloom to petal fall. Watch the model. After a period of warm weather, best results are obtained when antibiotics are applied within the 24-hour window before flower wetting during a high infection risk period. Products used must contact the interior of the flowers in sufficient water and approved wetting agent to completely cover the interior. Repeated antibiotic sprays may be necessary during extended high or

extreme risk periods. One pound of any 17% oxytetracycline product per 100 gallons gives a 200-ppm solution. Kasugamycin is another effective antibiotic. Some trials have shown that a full rate of Kasugamycin and a full rate of oxytetracycline provides excellent control. Applications of less than 100 gal/A can be effective on small trees if flower interiors are well covered, but do not drop the ppm below 200 (oxytetracycline). Application by ground equipment on each row is highly recommended (aircraft is NOT recommended). Many fire blight bacteria in the Pacific Northwest are resistant to streptomycin, another registered antibiotic.

Organic Management

Prebloom. Fixed copper sanitation if fire blight was in the ***orchard*** last year.

Early bloom. Lime sulfur plus oil (apples only). One to two applications of biologicals (Blossom Protect). Reapply biological after lime sulfur, which is antimicrobial.

Full bloom to petal fall. Depending on the cultivar russet risk and the CougarBlight model risk follow with *Bacillus subtilis* (Serenade Opti) (most fruit safe) every 2-5 days during flower/petal fall or copper hydroxide/octanoate (e.g. Cueva, Previsto) every 2 to 6 days (less fruit safe for russet). Coppers have had higher efficacy than biologicals during bloom in Washington trials. Do not follow coppers with any products with acidifiers. Good drying conditions are important to avoid russet risk.

Petal fall to two weeks after. Continue protective programs one to two weeks post petal fall. Warm conditions during late bloom increase fire blight risk for late blooms still present.

Strategies for Improving Protective Programs

Coverage. Product efficacy is based on thorough coverage of flowers. Use tree row volume to apply appropriate volumes to cover the tree architecture in your orchard. Products applied every other row or at high speeds may have insufficient coverage and lower efficacy.

Timing. Antibiotics have the highest efficacy when applied shortly before a moisture event. Nonetheless, Kasumamycin and Streptomycin can also be applied up to 12 hours after a moisture event, but with reduced effectiveness. Streptomycin has locally systemic activity and Kasumamycin is effective on bacteria which have been washed into the floral cup but not yet invaded the flower.

pH of spray tank water. It is important to appropriately acidify spray tank water when using antibiotics (especially oxytetraclycline and kasugamycin). Antibiotic efficacy reported in WSU trials is with spray tank water acidified to pH 5.6. At higher pH antibiotic degradation rate is higher and thus efficacy is often lower. For example, in one trial Kasugamycin reduced bacteria by 86 to 96% at pH 5.1 but only 21 to 35% at pH 7.3 (Adaskaveg, Forster, and Wade 2011).

Use appropriate rates. Quantity of active ingredient is important to obtain efficacy. For example, recent work looking at rates of copper products is demonstrating that as metallic copper content increases, copper product efficacy increases up to approximately 0.2 lb metallic copper per 100 gal per acre.

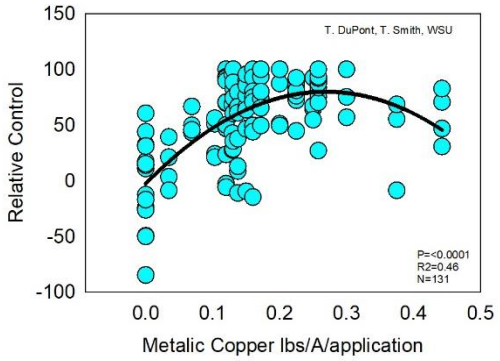

Figure 6 Relative control from coppers WSU trials 2013 to 2017.

Mixtures. A full rate of kasugamycin (100 ppm) with a full rate of oxytetracycline (200 ppm), as well as streptomycin (100 ppm) mixed with a full rate of oxytetracycline (200 ppm) have provided improved efficacy in some trials (Oregon 2015-2018).

Actigard (2oz) plus an antibiotic applied during bloom has improved the efficacy of antibiotics an average of 10% in trials in Washington and Oregon (Smith and Johnson 2011-2014).

Chemical Control Products

Biological Products

When applied to open flowers, these micro-organisms produce colonies on the stigma surfaces and nectary. With biological materials (e.g., Blossom Protect), spray treatments need to be initiated relatively early in the bloom period before high fire blight risk has developed.

Blossom Protect is a combination of two strains of *Aureobasidium pullulans*, a yeast that occurs naturally in Pacific Northwest pome fruit flowers. This organism grows on the nectary and stigmas of treated flowers and competes directing with the fire blight pathogen for the nutritional resource available on these surfaces. Blossom Protect is applied with a companion buffer, Buffer Protect, which reduces the pH of the sprayed suspension and helps the yeast grow faster than the pathogen. In Pacific Northwest trials, Blossom Protect has been the most effective bio-control organism to date (Johnson et al. 2014). If this product is used, it is important to spray every row at least once.

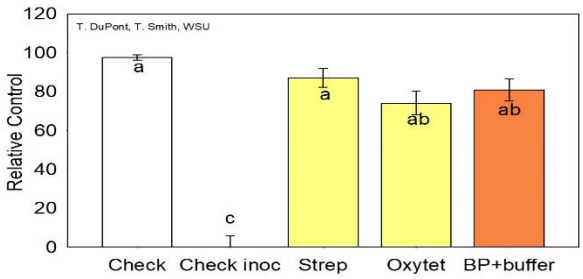

Figure 7 Blossom Protect in WSU Trials 2013, 2014, 2016, 2017.

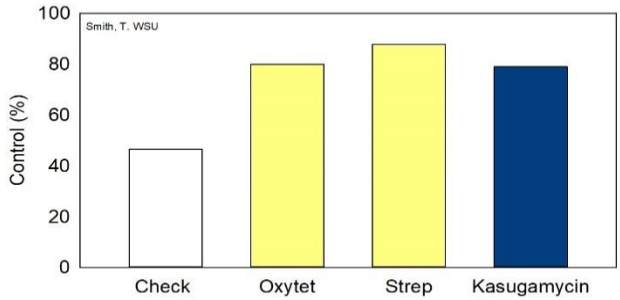

Figure 8 Percent control from antibiotics in WSU Trials 2006, 2009, 2010, 2011.

Antibiotics

Kasugamycin (tradename: Kasumin) is a recently labeled antibiotic that provides **good** levels of control (~80%). All *Erwinia amylovora* strains are currently sensitive to this material but there is an intermediate risk of resistance developing to this antibiotic (Adaskaveg, Forster, and Wade 2011). Kasumin controls streptomycin-resistant strains of *E. amylovora.* Kasumin provides forward control for two to four days prior to rain events (on flowers open when applied) and will be partially effective for blossom blight control if applied within 12 hours after a rain event. Kasumin is not locally systemic like streptomycin. Thus, Kasumin will not penetrate into the nectaries and will not be able to control an infection once the fire blight pathogen reaches the nectaries. Acidifying spray tanks (target 5) is important to reduce antibiotic break down and extend activity.

Oxytetracycline (tradenames: Mycoshield, FireLine) generally provide **good** levels of control in Washington trials and has a low risk of resistance development. Oxytetracycline products should be applied within one day prior to a rain event for best results. Oxytetracycline is considered bacteriostatic (inhibits bacterial growth). Thus, it has to be applied prior to rains where it can prevent growth on stigmas. Oxytetracycline is also sensitive to UV degradation and much of the activity is lost within one to two days after application. Acidifying spray tanks (target 5) is important to reduce antibiotic break down and extend activity.

Streptomycin (tradenames: Agri-Mycin, FireWall): Streptomycin-resistant strains of the fire blight pathogen have been present in Washington orchards since 1975 (Coyier and Covey 1975, Loper et al. 1991). Recent tests have indicated that the proportion of the pathogen population resistant to this antibiotic has dropped, and expected control levels have improved (Forster et al. 2015). This product should only be used in combination with oxytetracycline, and should not be used unless a high to extreme risk infection period is expected. Limit use to once per season. Remaining pathogen colonies in the orchard should be assumed to be streptomycin-resistant.

Coppers

Copper materials vary in the form and amount of metallic copper (the active ingredient). **"Fixed"** (copper hydroxides, copper oxychlorides) **-**copper products have a longer residual time and are generally used for delayed dormant (green tip) in bearing orchards and summer shoot blight protection in non-bearing (young) orchards. In fixed coppers, most of the copper is insoluble with soluble copper ions released slowly over time. Application of low-pH materials (e.g., Buffer Protect) to trees treated recently with a fixed copper can cause a large release of copper ions and increase the potential for phytotoxicity (Rosenberger 2011). Copper is toxic to plants when a sufficient concentration of ions penetrates tissue. Growers should avoid spray additives such as foliar nutrients and surfactants when applying coppers. Fixed-coppers should not be used with Imidan, Sevin, Thiodan, Captan, or phosophorus acid compounds (Fostphite, Prophyt, Phostrol, Agri-Fos, Alliete) (Shane and Sundin 2011).

Soluble coppers. Newer copper formulations are designed to reduce copper phytotoxicity and fruit russeting potential by introducing far few copper ions to the plant surface and adding safeners that also reduce injury potential. Examples are Cueva (copper octanoate), which is a salt of copper and a fatty acid (copper soap), and Previsto, which is copper ions in a matrix with alginate (polymer from seaweed). Both Cueva and Previsto have shown little phytotoxicity in semi-arid Washington trials but have shown some risk of russeting in wetter areas of Oregon and California. Cueva is compatible in tank-mixes with *Bacillus*-based biopesticides, while Previsto is not due to its high pH.

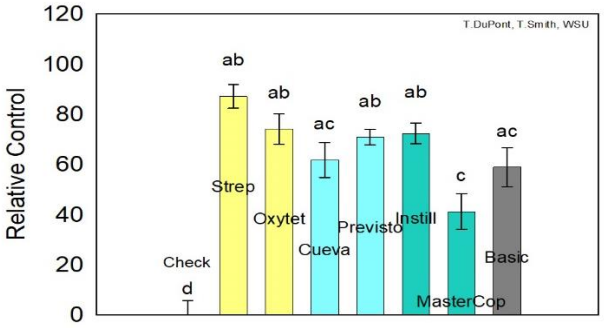

Figure 9 WSU trials 2013, 2014, 2016, 2017. Cueva (copper octanoate) 3-5 qt; Previsto (copper hydroxide) 3-5 qt; Instill (copper sulfate pentahydrate) 1-1.85 qrt; Mastercop copper sulfate pentahydrate) 0.25-1.25 qrt; Basic coppers Champ, Kocide (0.5 lb), Badge (1.25 pints). Rates/ 100 gal/Acre.

SARs

Acibenzolar-S-methyl (ASM, Actigard 50 WG), is a synthetic inducer of systemic acquired resistance (SAR). Its mode of action is to mimic the plant hormone, salicylic acid, which is responsible for priming the plant's defense system. The level of protection is smaller compared to an antibiotic but it lasts longer, approximately a week (Maxson-Stein et al. 2002).

Biorationals and Biopesticides

Serenade Optimum is an apparently 'fruit safe' material, made by fermenting a strain of *Bacillus subtilis*. The antimicrobial activity of Serenade comes primarily from biochemical compounds produced by the bacterium during fermentation, and not because of the bacterium's colonization of flowers in the orchard.

Apple Materials

Chemical	Rate per Acre	REI	PHI	MOA	Efficacy	Notes
Previsto *copper hydroxide*	3-4 qt	48 h	none listed		3	Pay attention to drying times and do not combine with acidifying products to reduce fruit finish risks.
Kasumin 2L *kasugamycin*	64 oz	12 h	90 d		4	Best control when applied less than 48 hrs before wetness event. Control up to 12 hr after wetness event.
DoubleNickel 55 *Bacillus amyloliquefaciens strain D747*	See label	4 h	0 d		2	See label and space between rows to select the corresponding rate. Efficacy may vary based on disease pressure.
Blossom Protect *Aureobasidium pullulans*	1.25 lb	4h	none listed		4	30 and 80% bloom. Yeasts need 1-2 days before an infection to colonize the flower before bacteria invade to be effective.
Cueva *copper octanoate*	4 qt	4 h	0 d		3	Little russet in semi-arid WA trials. Some russet risk in wetter OR. Tank mix compatible with Bacillus-based biopesticides.
FireLine 17WP *oxytetracycline*	See label	12 h	60 d		4	Best activity within 24 h before wetness event. Check spray tank pH, 5 optimal. 200 ppm: 1.0 lb/100 gal.
Mycoshield *oxytetracycline*	See Label	12 h	60 d		4	Best activity within 24 h before wetness event. Check spray tank pH, 5 optimal. 200 ppm: 1.0 lb/100 gal.
Actigard 50WG *acibenzolar-s-methyl*	2 fl oz	12 h	0 d			For bloom applications: Apply 2 oz/A in a tank mix with a fire blight treatment (generally an antibiotic) that is standard in your area. This is generally 2-3 applications between 20% bloom and petal fall depending on the environmental conditions. Do not apply closer than a 7-day interval.
NovaSource Lime Sulfur *lime sulfur/calcium polysulfide*	2 % v/v	48 h	none listed			Early bloom applications plus oil are antimicrobial. 20 and 70% bloom timings. Reapply biologicals after lime sulfur if used.
Serenade Opti *Bacillus subtilis strain QST 713*	20 oz	4 h	0 d			Efficacy may vary based on disease pressure.

Pear Materials

Chemical	Rate per Acre	REI	PHI	MOA	Efficacy	Notes
Kasumin 2L *kasugamycin*	64 fl oz	12 h	90 d		4	Best control when applied less than 48 hrs before wetness event. Control up to 12 hr after wetness event.
Actigard 50WG *acibenzolar-s-methyl*	2 fl oz	12 h	0 d			For bloom applications: Apply 2 oz/A in a tank mix with a fire blight treatment (generally an antibiotic) that is standard in your area. This is generally 2-3 applications between 20% bloom and petal fall depending on the environmental conditions. Do not apply closer than a 7-day interval.
Blossom Protect *Aureobasidium pullulans*	1.25 lb	4h	none listed		4	Apply with Buffer Protect. 30 and 80% bloom. Yeasts need 1-2 days before an infection to colonize the flower before bacteria invade to be effective. Russet potential on sensitive varieties in humid conditions.
FireLine 17WP *oxytetracycline*	1 lb	12 h	60 d		4	Best activity within 24 h before wetness event. Check spray tank pH, 5.5-6.0 optimal. 200 ppm: 1.0 lb/100 gal.
Previsto *copper hydroxide*	3-4 qt	48 h	none listed		3	Pay attention to drying times and do not combine with acidifying products to reduce fruit finish risks.
Serenade Opti *Bacillus subtilis strain QST 713*	20 oz	4 h	0 d			
Cueva *copper octanoate*	4 qt	4 h	0 d		3	Little russet in semi-arid WA trials. Some russet risk in wetter OR. Tank mix compatible with Bacillus-based biopesticides.
Mycoshield *oxytetracycline*	16 oz	12 h	60 d		4	Best activity within 24 h before wetness event. Check spray tank pH, 5.5-6.0 optimal. 200 ppm: 1.0 lb/100 gal.

Cutting Fire Blight Infections in Season

Cut hard, cut fast

An infected shoot has many millions to billions of pathogen cells. The highest concentration will be near to tip of the branch or infected floral cluster. By cutting a branch we hope to remove many of these cells so that they cannot flow through the tree where they may concentrate in other susceptible tissue and create new infections. Cut AT LEAST 12 to 18 inches below the noticeably infected area into two year or older wood in order to remove the highest concentration of pathogen cells. Young, vigorous or susceptible varieties will require cutting further. Removing infected tissue quickly increases the likelihood of removing more pathogen cells before they invade deeply into the tree. Some recommendations suggest an 'ugly stub cut' where growers make cuts 20-30 cm below visible symptoms into two-year-old or older wood (where resistance is greater due to carbohydrate reserves (Suleman and Steiner 1994) leaving a 10 to 12 cm naked stub. While small cankers will form on many of these cuts, these cankers can be removed during winter pruning (Steiner 2000).

Use of concentrated Actigard during blight clean-up

New research has shown that treatment of trees with the chemical, Acibenzolar-S-methyl (ASM, Actigard 50 WG), may reduce re-occurrence of blight after cutting out infected strikes. Re-occurrence happens when the act of cutting out the disease does not completely remove the pathogen cells that have moved ahead of the expanding canker.

Plants have defense systems. If something stimulates the plant's defense response before the symptoms develop (or re-develop), the plant will be in an active defense mode and will be less affected by disease when it occurs (or re-occurs). Actigard is a compound that has been found to trigger induced resistance. Its mode of action is to mimic the plant hormone, salicylic acid, which is responsible for priming the plant's defense system.

For more than five years, Dr. Ken Johnson of Oregon State University has found that painting a concentrated solution of Actigard on trees after cutting out infection reduced the severity of re-occurring fire blight cankers in pears. For example, he found that without treatment after cutting out fire blight cankers in young Bosc pear trees, the disease came back 50% of the time and began to run through the tree. With Actigard applications, both the proportion of trees in which fire blight came back and the rate of canker expansion was reduced (Johnson and Temple 2016).

During the summer, cut out blight when you see it. Removing a strike can greatly reduce further damage on the tree, especially if you catch the strike early. Apply concentrated Actigard with an up and down motion to a ½ meter length of the central leader or major scaffold near where the fire blight infection was removed. Use the labeled rate of 1 oz/ 1 quart with 1% silicone-based penetrant. One quart will treat approximately 500 cuts.

Additional Resources

Decision Aid System
Visit for the recent model projections of blossom blight risk at your site.

Crop Protection Guide
Crop Protection Guide recommendations are updated on an annual basis.

Organic Fire Blight Management in the Western US
https://articles.extension.org/pages/74505/organic-fire-blight-management-in-the-western-us

Dealing with Fire Blight Once it is in the Orchard.
WSU Newsletter article July 2017.

Tips for Using Blossom Protect
WSU Newsletter article April 10, 2017.

Remember last year's infections are this year's risk.
WSU Newsletter article April 2018.

Canker Management
WSU Newsletter article January 2019.

Use pesticides with care. Apply them only to plants, animals, or sites listed on the labels. When mixing and applying pesticides, follow all label precautions to protect yourself and others around you. It is a violation of the law to disregard label directions. If pesticides are spilled on skin or clothing, remove clothing and wash skin thoroughly. Store pesticides in their original containers and keep them out of the reach of children, pets, and livestock.

YOU ARE REQUIRED BY LAW TO FOLLOW THE LABEL. It is a legal document. Always read the label before using any pesticide. You, the grower, are responsible for safe pesticide use. Trade (brand) names are provided for your reference only. No discrimination is intended, and other pesticides with the same active ingredient may be suitable. No endorsement is implied.

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Kasumin™ 2L

Bactericide

Appendix B

GROUP 24 BACTERICIDE

COMMERCIAL
LIQUID

READ THE LABEL BEFORE USING

KEEP OUT OF REACH OF CHILDREN
POTENTIAL SENSITIZER

GUARANTEE:
Kasugamycin, present as hydrochloride hydrate. 2.00%
Contains 1,2-benzisothiazolin-3-one at 0.02% as a preservative

REGISTRATION NO. 30591 **PEST CONTROL PRODUCTS ACT**

In case of emergency involving a major spill, fire or poisoning call
CHEMTREC 24-hours at 703-527-3887 or 1-800-424-9300

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Arysta LifeScience

GENERAL INFORMATION

Kasumin 2L Bactericide is a liquid formulation of kasugamycin hydrochloride hydrate (2.3%) containing 2 % (by weight) of kasugamycin. Kasugamycin is an aminoglycosidic antibiotic (bactericide) that controls fire blight (*Erwinia amylovora*) on pome fruit and suppresses walnut blight (*Xanthomonas campestris* pv. *juglandis*) on walnuts as well as bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial stem canker (*Clavibacter michiganensis* subsp. *michiganensis*) on fruiting vegetables (greenhouse and field).

PRECAUTIONS

- **KEEP OUT OF REACH OF CHILDREN**
- May cause sensitization.
- Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

Wear a long-sleeved shirt and long pants, chemical-resistant gloves, socks and shoes during mixing, loading, application, clean-up and repair. It is important to wear gloves for the mixing/loading operation and when making sprayer and nozzle repairs and adjustments. Do not handle this product with bare hands. Follow the manufacturer's instructions for cleaning/maintaining personal protective equipment. If no such instructions for washables are available, use detergent and hot water. Keep and wash personal protective equipment separately from other laundry.

Change contaminated clothing daily and wash before use. Remove clothing immediately if pesticide gets inside. Shower immediately and put on clean clothing. Remove PPE immediately after handling this product. Wash the outside of gloves and leave on before removing any protective clothing. As soon as possible shower and change into clean clothing.

Apply only when the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools and recreational areas is minimal. Take into consideration wind speed, wind direction, temperature inversions, application equipment and sprayer settings.

RESTRICTED ENTRY INTERVAL: DO NOT enter or allow worker entry into treated areas (including greenhouses where crops have been treated) during the restricted-entry interval (REI) of 12 hours following application.

Note: If this pest control product is to be used on a commodity that may be exported to the US and you require information on acceptable residue levels in the US, visit CropLife Canada's web site at: www.croplife.ca.

DO NOT apply this product by air. Use only according to label directions.

FIRST AID	
If in eyes	Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing. Call a poison control centre or doctor for treatment advice.
If on skin or clothing	Take off contaminated clothing. Rinse skin with plenty of water for 15–20 minutes. Call a poison control centre or doctor for treatment advice.
If inhaled	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.
If swallowed	Call a poison control centre or doctor for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person.
Take container, label or product name and Pest Control Product Registration Number with you when seeking medical attention.	

In case of emergency involving a major spill, fire or poisoning call CHEMTREC 24-hours at 703-527-3887 or 1-800-424-9300

TOXICOLOGICAL INFORMATION

No specific antidote available. Treat symptomatically.

ENVIRONMENTAL HAZARDS

Toxic to non-target terrestrial plants. Observe buffer zones specified under DIRECTIONS FOR USE.

To reduce runoff from treated areas into aquatic habitats avoid application to areas with a moderate to steep slope, compacted soil, or clay.

Avoid application when heavy rain is forecast.

Contamination of aquatic areas as a result of runoff may be reduced by including a vegetative strip between the treated area and the edge of the water body.

STORAGE AND DISPOSAL

STORAGE: Store in a dry place away from excessive heat. To prevent contamination store this product away from food or feed. Store in original container only.

DISPOSAL:

DO NOT reuse this container for any purpose. This is a recyclable container, and is to be disposed of at a container collection site. Contact your local distributor/dealer or municipality for the location of the nearest collection site. Before taking the container to the collection site:

1. Triple- or pressure-rinse the empty container. Add the rinsings to the spray mixture in the tank.
2. Make the empty, rinsed container unsuitable for further use.

If there is no container collection site in your area, dispose of the container in accordance with provincial requirements.

For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of a spill, and for clean-up of spills.

DIRECTIONS FOR USE

This product contains the bactericide kasugamycin. To reduce the development of resistant plant pathogenic bacteria, this product should be used only when required.

As this product is not registered for the control of pests in aquatic systems, DO NOT use to control aquatic pests.

DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.

Field sprayer application: **DO NOT** apply during periods of dead calm. Avoid application of this product when winds are gusty. **DO NOT** apply with spray droplets smaller than the American Society of Agricultural Engineers (ASAE) medium classification. Boom height must be 60 cm or less above the crop or ground.

Airblast application: **DO NOT** apply during periods of dead calm. Avoid application of this product when winds are gusty. **DO NOT** direct spray above plants to be treated. Turn off outward pointing nozzles at row ends and outer rows. **DO NOT** apply when wind speed is greater than 16 km/h at the application site as measured outside of the treatment area on the upwind side.

DO NOT apply by air.

Buffer zones:

Use of the following spray methods or equipment **DO NOT** require a buffer zone: hand-held or backpack sprayer and spot treatment.

The buffer zones specified in the table below are required between the point of direct application and the closest downwind edge of sensitive terrestrial habitats (such as grasslands, forested areas, shelter belts, woodlots, hedgerows, riparian areas and shrublands).

Method of application	Crop		Buffer Zones (metres) Required for the Protection of Terrestrial habitat:
Airblast	Pome fruit and walnuts	Early growth stage	2

For tank mixes, consult the labels of the tank-mix partners and observe the largest (most restrictive) buffer zone of the products involved in the tank mixture and apply using the coarsest spray (ASAE) category indicated on the labels for those tank mix partners.

APPLICATION GUIDELINES

Broadcast Ground Sprayers

Thorough coverage is necessary to provide good disease control. Applications using sufficient water volume to provide thorough and uniform coverage generally provide the most effective disease control. Check the sprayer frequently to ensure proper calibration and continued uniform application. To avoid streaked, uneven or overlapped application, use appropriate marking devices.

Use Precautions

Read and understand the entire label before opening this product. If you have questions, call Arysta LifeScience North America, LLC at 1-866-761-9397 or obtain technical advice from the distributor or your provincial agricultural representative. Application of KASUMIN 2L BACTERICIDE must meet and or conform to the following:

USE DIRECTIONS FOR SPECIFIC CROPS

KASUMIN 2L BACTERICIDE provides control or suppression of important diseases of fruiting vegetables, pome fruit, and walnuts.

FRUITING VEGETABLES—CROP GROUP 8 (GREENHOUSE OR FIELD):
Eggplant, Groundcherry, Pepino, Pepper (includes bell pepper, chili pepper, cooking pepper, pimento, sweet pepper), Tomatillo, Tomato

Disease Suppression	Application Rate	Application Timing and Resistance Management
Bacterial Spot (*Xanthomonas campestris* pv. *vesicatoria*) **Bacterial Stem Canker** (*Clavibacter michiganensis* subsp. *michiganensis*)	1.2L/ha	• Spray volume must be sufficient to provide good coverage of treated foliage. • Begin applications when conditions favour disease development. • Repeat applications at intervals that are necessary or when conditions favour disease development.

RESTRICTIONS AND OTHER INFORMATION:

- Do not apply more than 3.6 L Kasumin 2L Bactericide per hectare per year.
- Do not make more than 3 applications of Kasumin 2L Bactericide per season.
- A minimum interval of 7 days between applications is required.
- Do not make more than two consecutive applications of Kasumin 2L Bactericide. If additional applications are needed, rotate with another product with a different mode of action that is registered for this use.
- For resistance management purposes, do not apply on greenhouse vegetable transplants.
- Do not apply Kasumin 2L Bactericide within 1 day of harvest.

TANK MIXES

Kasumin 2L Bactericide may be tank-mixed with Kocide DF Fungicide/Bactericide (PCP# 24538), Kocide 101 Fungicide (PCP# 14417), or Kocide 2000 (PCP# 27348) for control of registered bacterial diseases on tomatoes and peppers (greenhouse and field). When applied as a tank-mix combination, read and observe all label directions, including rates, and restrictions for each product used in the tank-mix. Follow the more stringent label precautionary measures for mixing, loading and applying stated on both product labels.

POME FRUIT—CROP GROUP 11-09 (BEARING AND NONBEARING):
Apple, Azarole, Crabapple, Mayhaw Medlar, Pear, Asian Pear, Quince, Chinese Quince, Japanese Quince, Tejocote, Cultivars, varieties and/or hybrids of these commodities

Disease Control	Application Rate	Application Timing and Resistance Management
Fire Blight (*Erwinia amylovora*)	5.0 L/ha	• Spray volume must be sufficient to provide good coverage of treated foliage. • Reduced spray volumes may be utilized for small trees where complete coverage can be obtained with less water per hectare. • Begin applications at 20–30% bloom or when conditions favour disease development. • Repeat applications at 7-day intervals or when conditions favour disease development.

RESTRICTIONS AND OTHER INFORMATION:

- Do not apply more than 20.0 L of Kasumin 2L Bactericide per hectare per year.
- Do not make more than 4 applications of Kasumin 2L Bactericide per season.
- Do not make more than two consecutive applications of Kasumin 2L Bactericide. If additional applications are needed, rotate with another product with a different mode of action that is registered for this use.
- Do not use alternate tree-row application method.
- Do not apply after petal fall.
- Do not apply Kasumin 2L Bactericide within 90 days of harvest.

WALNUT		
Disease Suppression	**Application Rate**	**Application Timing and Resistance Management**
Walnut Blight (*Xanthomonas campestris* pv. *juglandis*)	5.0 L/ha	• Spray volume must be sufficient to provide good coverage of treated foliage. • Reduced spray volumes may be utilized for small trees where complete coverage can be obtained with less water per hectare. • Begin applications when conditions favour disease development.

RESTRICTIONS AND OTHER INFORMATION:

- Do not apply more than 20.0 Litres of Kasumin 2L Bactericide per hectare per year.
- Do not make more than 4 applications of Kasumin 2L Bactericide per season.
- A minimum interval of 14 days between applications is required.
- Do not make more than two consecutive applications of Kasumin 2L Bactericide. If additional applications are needed, rotate with another product with a different mode of action that is registered for this use.
- Do not use alternate tree-row application method
- Do not apply Kasumin 2L Bactericide within 100 days of harvest.

MIXING PROCEDURES

Prepare no more spray mixture than is needed for the immediate operation. Thoroughly clean spray equipment before using this product. Agitation is necessary for proper dispersal of the product. Maintain maximum agitation throughout the spraying operation. Do not let the spray mixture stand overnight in the spray tank. Flush the spray equipment thoroughly following each use and apply the rinsate to a previously treated area.

If using KASUMIN 2L BACTERICIDE in a tank-mixture with Kocide DF, Kocide 101 or Kocide 2000, observe all directions for use, crop/sites, use rates, dilution ratios, precautions, and limitations, which appear on the Kocide product label. No label dosage rate may be exceeded, and the most restrictive label precautions and limitations must be followed.

RESISTANCE MANAGEMENT RECOMMENDATIONS

For resistance management, please note that Kasumin 2L Bactericide contains a Group 24 bactericide. Any microbial population may contain individuals naturally resistant to Kasumin 2L Bactericide and other Group 24 Bactericides. A gradual or total loss of pest control may occur over time if these bactericides are used repeatedly in the same fields. Other resistance mechanisms that are not linked to the site of action but specific for individual chemicals, such as enhanced metabolism, may also exist. Appropriate resistance-management strategies should be followed. To reduce the likelihood of bacteria developing resistance to kasugamycin, sound resistance management practices should be employed when using this product. Such practices include limiting the number of consecutive applications of Kasumin 2L Bactericide, and alternating Kasumin 2L Bactericide applications with other bactericides that have a different mode of action. See crop specific use directions above.

To delay resistance:

- Where possible, rotate the use of Kasumin 2L Bactericide with products from different groups that control the same pathogens.
- Do not make more than two consecutive applications of Kasumin 2L Bactericide before alternating to a registered bactericide with a different mode of action.
- Use tank-mixtures with bactericides from a different group when such use is permitted.
- Kasumin 2L Bactericide use should be based on an IPM program that includes scouting, historical information related to pesticide use and crop rotation and considers cultural, biological, and other chemical control practices.
- Monitor treated pathogen populations for resistance development.
- If disease continues to progress after treatment with this product, do not increase the use rate. Discontinue use of this product, and switch to another bactericide with a different target site of action, if available.
- Contact your local extension specialist or certified crop advisors for any additional pesticide resistance-management and/or IPM recommendations for specific crops and pathogens.
- For further information and to report suspected resistance, contact Arysta LifeScience North America LLC at 1-866-761-9397.

NOTICE TO USER

This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *PEST CONTROL PRODUCTS ACT* to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any such use of this product.

GROUP 24 BACTERICIDE
GROUPE 24 BACTÉRICIDE

Kasumin™ 2L

Bactericide / Bactéricide

COMMERCIAL
LIQUID

READ THE LABEL BEFORE USING

KEEP OUT OF REACH OF CHILDREN
POTENTIAL SENSITIZER

GUARANTEE:

Kasugamycin, present as hydrochloride hydrate 2.00%
Contains 1,2-benzisothiazolin-3-one at 0.02% as a preservative

REGISTRATION NO. 30591
PEST CONTROL PRODUCTS ACT

FIRST AID	
If in eyes	Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing. Call a poison control centre or doctor for treatment advice.
If on skin or clothing	Take off contaminated clothing. Rinse skin with plenty of water for 15–20 minutes. Call a poison control centre or doctor for treatment advice.
If inhaled	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.
If swallowed	Call a poison control centre or doctor for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person.
Take container, label or product name and Pest Control Product Registration Number with you when seeking medical attention.	

In case of emergency involving a major spill, fire or poisoning call CHEMTREC 24-hours at 703-527-3887 or 1-800-424-9300

Arysta LifeScience North America LLC
15401 Weston Parkway, Suite 150
Cary, NC 27513 USA
AD021513 • 103135—022513
EPA Est. No. 70815-GA-001
For Product Use Information Call 1-866-761-9397
Renseignements sur le produit : 1-866-761-9397

COMMERCIAL
LIQUIDE

LIRE L'ÉTIQUETTE AVANT L'EMPLOI

GARDER HORS DE LA PORTÉE DES ENFANTS
SENSIBILISANT CUTANÉ POTENTIEL

GARANTIE :

Kasugamycine, présente sous forme d'hydrate d'hydrochlorure 2,00%
Contient du 1,2-benzisothiazoline-3-one à raison de 0,02 % à titre d'agent de conservation

N° D'HOMOLOGATION 30591
LOI SUR LES PRODUITS ANTIPARASITAIRES

PREMIERS SOINS	
En cas de contact avec les yeux	Garder les paupières écartées et rincer doucement et lentement avec de l'eau pendant 15 à 20 minutes. Le cas échéant, retirer les lentilles cornéennes au bout de 5 minutes et continuer de rincer l'œil. Appeler un centre antipoison ou un médecin pour obtenir des conseils sur le traitement.
En cas de contact avec la peau ou les vêtements	Enlever tous les vêtements contaminés. Rincer immédiatement la peau à grande eau pendant 15 à 20 minutes. Appeler un centre antipoison ou un médecin pour obtenir des conseils sur le traitement.
En cas d'inhalation	Déplacer la personne vers une source d'air frais. Si la personne ne respire pas, appeler le 911 ou une ambulance, puis pratiquer la respiration artificielle, de préférence le bouche-à-bouche, si possible. Appeler un centre antipoison ou un médecin pour obtenir des conseils sur le traitement.
En cas d'ingestion	Appeler un centre antipoison ou un médecin immédiatement pour obtenir des conseils sur le traitement. Faire boire un verre d'eau à petites gorgées si la personne empoisonnée est capable d'avaler. Ne pas faire vomir à moins d'avoir reçu le conseil de procéder ainsi par le centre antipoison ou le médecin. Ne rien administrer par la bouche à une personne inconsciente.
Emporter le contenant, l'étiquette ou prendre note du nom du produit et de son numéro d'homologation lorsqu'on cherche à obtenir une aide médicale.	

En cas d'urgence relativement à un déversement, un incendie ou un empoisonnement d'importance, appeler CHEMTREC 24 heures par jour au 703-527-3887 ou au 1-800-424-9300

NET CONTENTS: 10 L
CONTENU NET : 10 L

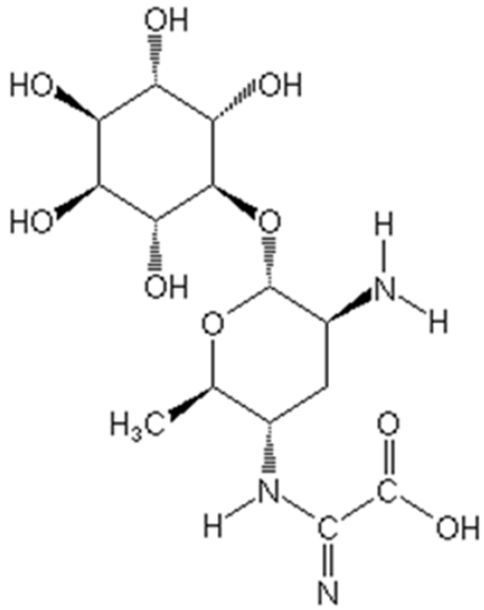
Kasumin®

A valuable and essential tool for controlling bacterial diseases; suppressing pathogen growth before disease spreads.

Kasumin®

Product Information

- **Active ingredient** – kasugamycin
- **Formulation:** 2% SL
- **Signal Word:** CAUTION
- **Synthesized by fermenting *Streptomyces kasugaensis***
- **Mode of Action:** FRAC 24 - Aminoglycoside antibiotic
  - Blocks protein formation in bacterial ribosomes, shutting down energy production of the target pathogen



UPL

Always read and follow label directions.

Kasumin®

Product Information

- **High level of preventative activity**
- **Systemic in foliage and green tissue**
- **Residual:** ~ 7 days
- **Rainfast:** After 1 hour
- **No observations of human or animal cross resistance as with other antibiotic options**
  - No human or animal uses of kasugamycin
  - Ongoing resistance monitoring in the field as required by EPA has not show any signs of cross resistance
- **No cross resistance to Streptomycin or other bactericides**
  - Will control streptomycin and copper resistant strains of bacteria
- **ONLY active ingredient in FRAC group 24**

UPL

Always read and follow label directions.

Product Information

- **Highly effective against bacterial blight diseases in labeled crops.**
  - **Apple & Pear –** Fire Blight
  - **Walnuts –** Walnut Blight
  - **Cherries –** Bacterial Blast & Canker
- **No Phytotoxicity has been observed following Kasumin applications**
- **Does not pose a risk to bees when applied according to the label**
  - Kasumin bee toxicity was tested for both oral and contact exposure

UPL **Always read and follow label directions.**

United States
Environmental Protection
Agency

Office of Prevention, Pesticides
and Toxic Substances
(7501C)

Pesticide Fact Sheet

Appendix C

Name of Chemical: Kasugamycin
Reason for Issuance: New Chemical
Tolerance Established
Date Issued: September 2005

Description of Chemical

Generic Name:	3-O-[2-amino-4-[(carboxyiminomethyl) amino]-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl]-D-chiro-inositol
Common Name:	Kasugamycin
Trade Name:	Kasumin® 2L
Chemical Class:	Aminoglycoside Antibiotic Fungicide
EPA Chemical Code:	230001
Chemical Abstracts Service (CAS) Number:	6980-18-3
Registration Status:	Not Registered, Import Tolerance Established
Pesticide Type:	Fungicide
U.S. Agent:	Arysta Lifescience North American Corporation (*Formerly known as Arvesta Corporation*) 100 First Street, Ste. 1700 San Francisco, CA 94105
International Producer:	Hokko Chemical Industry Co. Ltd. 4-2 Nihonbashi Hongoku-Cho, Chuo-Ku Tokyo, Japan

Tolerances Established

Tolerances were established in the 40 CFR §180.614 for Vegetable, Fruiting Group 8 at 0.04 ppm.

Use Pattern and Formulations

Kasugamycin is not registered in the U.S., however, tolerances were established to cover residues on imported tomatoes and peppers from Mexico where the registrant is seeking to register Kasumin® 2L, a liquid formulation comprised of 2% kasugamycin (by weight) as the active ingredient (ai), for use on rice, potato, pepper, and tomato in Mexico. The product is formulated from kasugamycin hydrochloride hydrate (2.3%) and contains 2% kasugamycin (0.1667 lb ai/gal) as the free base. Kasugamycin is also formulated as a co-active ingredient (at 5%) along with copper oxychloride (at 45%, expressed as copper) in a wettable powder (WP), designated Kasumin Cobre®. Additionally, the Kasumin® formulation is a WP containing 8% kasugamycin. The principal target organisms are bacteria rot (*Erwinia atroseptica*) and leaf mold (*Cladosporium fulvum*) on tomato, and bacteria spot (*Xanthomonas campestris*, pv *vesicatoria*) on both tomato and pepper.

TABLE 1 Summary of Directions for Use of Kasugamycin in Mexico.

Trade Name Formulation [EPA Reg. Number]	Application Type/Timing and Equipment	Application Rate (lb ai/A)	Maximum Number of Applications per Season	Maximum Seasonal Application Rate (lb ai/A)	PHI (Days)	Use Directions and Limitations
Proposed Use on Fruiting Vegetables (Group 8) Imported from Mexico						
Kasumin® 2L [None]	Foliar broadcast/ none specified	0.018	3	0.054	1	None specified

Science Findings

Available product chemistry and toxicology data supporting the proposed tolerance are summarized below.

Physical/Chemical Structure:

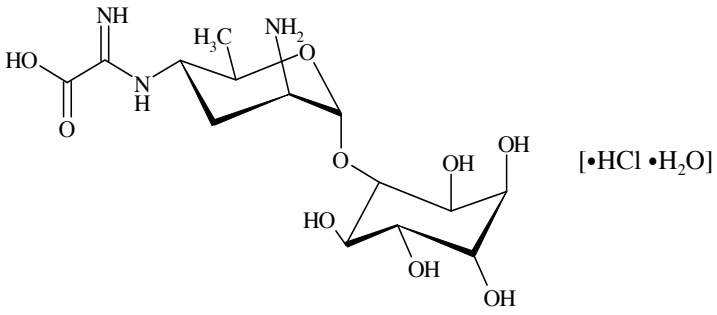


TABLE 2 Physicochemical Properties of the Technical Grade Compound (Kasugamycin Hydrochloride Hydrate).		
Parameter	**Value**	**Reference**
Molecular Weight	433.8	MRIDs # 45910004 and -05
Melting Point/Range	202-230°C (decomposing)	
pH	4.35 at 24.5°C (1% wt/vol solution)	
Density	0.43 g/mL at 24.5°C	
Water Solubility	g/100 mL pH 5 20.7 pH 7 22.8 pH 9 43.8	
Solvent Solubility	g/100 mL Methanol 0.744 Hexane $<1 \times 10^{-5}$ Acetonitrile $<1 \times 10^{-5}$ Methylene chloride $<1 \times 10^{-5}$	
Vapor Pressure	<0.013 mPa at 25°C	
Dissociation Constant (pK_a)	$pK_{a1} = 3.23$ $pK_{a2} = 7.73$ $pK_{a3} = 11.0$	
Octanol/Water Partition Coefficient (Log [K_{OW}])	<1.96 at 23°C and pH 5	
UV/Visible Absorption Spectrum	Not available	

TABLE 3 Acute Toxicity Profile for Kasugamycin.					
Test Material* [% ai]	**Guideline Number**	**Study Type**	**MRID Number**	**Results**	**Toxicity Category**
Technical Product [71]	**870.1100**	Acute oral - rat	45910012	LD_{50} (♂+♀) > 5000 mg/kg	IV
End-Use Product (EP) [2.0]	**870.1100**	Acute oral - rat	45910014	LD_{50} (♂+♀) > 5000 mg/kg	IV
EP [2.0]	**870.1100**	Acute oral - mouse	45910013	LD_{50} (♂+♀) > 5000 mg/kg	IV
EP [2.0]	**870.1200**	Acute dermal - rat	46030301	LD_{50} (♂+♀) > 2000 mg/kg	III
EP [2.2]	**870.1300**	Acute inhalation - rat	45910018	LC_{50} (♂+♀) > 4.892 mg/L	IV
EP [2.0]	**870.2400**	Acute eye irritation - rabbit	45910015	Mild eye irritant (iritis at 1 hour, resolving by 24 hours; conjunctivitis at 1 hour, resolving by 24 hours).	IV
EP [2.0]	**870.2500**	Acute dermal irritation - rabbit	45910017	Not irritating to the skin.	IV
EP [2.0]	**870.2600**	Skin sensitization - guinea pig	45910016	Not a sensitizer under the conditions of this study.	Not applicable

* Bracketed values are % ai as kasugamycin free base.

TABLE 4 Subchronic, Chronic, and Other Toxicity Profile for Kasugamycin.				
Guideline Number	**Study Type/ *Classification***	**MRID Number**	**Doses**	**Results**
870.3100	90-Day oral toxicity rodents - rat *Acceptable/guideline*	45910020	0, 300, 1000, 3000, 6000 ppm **M:** 0, 17.5, 58.2, 176.7, 354.8 mg/kg/day **F:** 0, 20.3, 69.2, 201.0, 395.5 mg/kg/day	**NOAEL** = 176.7/201.0 mg/kg/day (M/F) **LOAEL** = 354.8/395.5 mg/kg/day (M/F) based on decreased body weights and body weight gains.
870.3100	90-Day oral toxicity rodents - mouse *Acceptable/guideline*	45910019	0, 300, 1000, 3000, 10000 ppm **M:** 0, 41.2, 135.4, 408.5, 1559 mg/kg/day **F:** 0, 58.0, 170.9, 565.6, 1834 mg/kg/day	**NOAEL** = 135.4/170.9 mg/kg/day (M/F) **LOAEL** = 408.5/565.6 mg/kg/day (M/F) based on increased mortality and anal lesions (M&F), and kidney lesions (F). At 1559/1834 mg/kg/day (M/F), decreased body weights and body weight gains (M&F), testicular tubular dilatation and degeneration, perianal/perigenital staining (F), and extramedullary hematopoiesis of the spleen (M) were seen.
870.3150	90-Day oral toxicity in nonrodents - dog *Acceptable/guideline*	46030302	0, 300, 3000, 6000/0/4500* ppm **M:** 0, 10.6, 106.0, 182 mg/kg/day **F:** 0, 11.4, 107.9, 179 mg/kg/day * The high-dose group was exposed to 6000 ppm on weeks 1-5, control diet on weeks 6-8, and 4500 ppm on weeks 8-13.	**NOAEL** = 10.6/11.4 mg/kg/day (M/F) **LOAEL** = 106.0/107.9 mg/kg/day (M/F) based on tongue lesions, few feces, swollen mouth, excessive salivation, and thickened skin at the commissure of the mouth. At 182/170 mg/kg/day (M/F), decreased body weights, body weight gains, and food consumption were seen.

TABLE 4 Subchronic, Chronic, and Other Toxicity Profile for Kasugamycin.				
Guideline Number	**Study Type/ *Classification***	**MRID Number**	**Doses**	**Results**
870.3700	Pre-natal developmental in rodents - rat *Acceptable/guideline*	45910022	0, 40, 200, 1000 mg/kg/day	**Maternal** **NOAEL** = 200 mg/kg/day **LOAEL** = 1000 mg/kg/day based on decreased body weights, body weight gains, and food consumption; increased incidence of loose stool; and distention of the large intestine with stool in the cecum. **Developmental** **NOAEL** = 1000 mg/kg/day **LOAEL** = >1000 mg/kg/day
870.3700	Pre-natal developmental in nonrodents - rabbit *Acceptable/guideline*	46030303	0, 1, 3, 10 mg/kg/day	**Maternal** **NOAEL** = 10 mg/kg/day **LOAEL** = >10 mg/kg/day Note: Abortions and decreased maternal body weights, body weight gains, and food consumption were seen at 30 mg/kg/day in a range-finding study. **Developmental** **NOAEL** = 10 mg/kg/day **LOAEL** = >10 mg/kg/day

TABLE 4 Subchronic, Chronic, and Other Toxicity Profile for Kasugamycin.				
Guideline Number	**Study Type/ *Classification***	**MRID Number**	**Doses**	**Results**
870.3800	Reproduction and fertility effects - rat *Acceptable/guideline*	45910023	0, 200, 1000, 6000 ppm **M:** 0, 13.7, 70.3, 425.3 mg/kg/day **F:** 0, 16.2, 82.9, 503.4 mg/kg/day	**Parental/Systemic** **NOAEL** = 13.7/16.2 mg/kg/day (M/F) **LOAEL** = 70.3/82.9 mg/kg/day (M/F) based on decreased body weights and body weight gains. At 425.3/503.4 mg/kg/day (M/F), red and swollen skin around the anal opening (M&F) and testicular atrophy/degeneration in F1 males were seen. **Reproductive** **NOAEL** = 70.3/82.9 mg/kg/day (M/F) **LOAEL** = 425.3/503.4 mg/kg/day (M/F) based on decreased fertility and fecundity in the F1 parents for both litters and increased pre-coital interval during the mating period for the F2 litter. **Offspring** **NOAEL** = 425.3/503.4 mg/kg/day (M/F) **LOAEL** = >425.3/503.4 mg/kg/day (M/F)
870.4100	Chronic toxicity - rodents			See 870.4300. This study includes requirements of both 870.4100 and 870.4200.
870.4100	Chronic toxicity - dog *Acceptable/guideline*	46185901	0, 300, 1000, 3000 ppm **M:** 0, 10.5, 30.5, 99.6 mg/kg/day **F:** 0, 9.4, 33.4, 103.6 mg/kg/day	**NOAEL** = 99.6/103.6 mg/kg/day (M/F) **LOAEL** = >99.6/103.6 mg/kg/day (M/F)
870.4200	Carcinogenicity - rat			See 870.4300. This study includes requirements of both 870.4100 and 870.4200.
870.4200	Carcinogenicity - mouse *Acceptable/guideline*	46030304	0, 50, 300, 1500 ppm **M:** 0, 5.93, 34.94, 186.3 mg/kg/day **F:** 0, 7.25, 42.29, 215.2 mg/kg/day	**NOAEL** = 186.3/215.2 mg/kg/day (M/F) **LOAEL** = >186.3/215.2 mg/kg/day (M/F) *No evidence of carcinogenicity*

TABLE 4 Subchronic, Chronic, and Other Toxicity Profile for Kasugamycin.				
Guideline Number	**Study Type/ *Classification***	**MRID Number**	**Doses**	**Results**
870.4300	Combined chronic toxicity/ carcinogenicity - rat *Acceptable/guideline*	45910024	0, 30, 300, 3000 ppm **M:** 0, 1.1, 11.3, 116 mg/kg/day **F:** 0, 1.4, 13.4, 140 mg/kg/day	**NOAEL** = 11.3/140 mg/kg/day (M/F) **LOAEL** = 116/>140 mg/kg/day (M/F) based on increased testicular softening and atrophy in males. *No evidence of carcinogenicity*
870.5100	Gene mutation - bacterial reverse mutation assay *Unacceptable/ upgradable*	45910028	0, 5, 10, 50, 100, 500 ug/plate for *Salmonella typhimurium* strain G46 (his^-) 0, 5, 10, 50, 100, 200 ug/plate for all other strains tested	No mutagenic activity in bacteria (*Salmonella typhimurium* and *Escherichia coli*) under conditions of this assay. Not tested up to the limit dose, no indication of cytotoxicity, and no defined limit of solubility.
870.5300	Cytogenetics - *in vitro* mammalian cell gene mutation test (CHO Cells) *Acceptable/guideline*	45910026	0, 0.5, 1, 2, 4, 6, 8, 10 mg/ml	No increase in mutant frequency at the HGPRT locus, in the presence or absence of S9 activation.
870.5375	Cytogenetics - *in vitro* mammalian cell chromosome aberration test *Unacceptable/not upgradable*	45910025	0, 1, 2, 3, 4, 5 mg/ml	No increase in mutant frequency, in the presence or absence of S9 activation. The time from treatment to cell harvest was insufficient.
870.5395	Cytogenetics - mammalian erythrocyte micronucleus test (mice) *Acceptable/guideline*	46030305	0, 200, 1000, 5000 mg/kg	No evidence of induced chromosomal damage or other damage leading to micronucleus formation.
870.5550	Other effects - unscheduled DNA synthesis in mammalian cells in culture (rats) *Acceptable/guideline*	45910027	First assay: 0-2.5 mg/ml Second assay: 0-10 mg/ml Third assay: 0-10 mg/ml	No evidence that unscheduled DNA synthesis was induced.

TABLE 4	Subchronic, Chronic, and Other Toxicity Profile for Kasugamycin.			
Guideline Number	**Study Type/ *Classification***	**MRID Number**	**Doses**	**Results**
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/guideline*	46030306	**(1)** 100 mg/kg radiolabeled, single dose by oral gavage. **(2)** 100 mg/kg unlabeled, 14 days in the diet, PLUS 100 mg/kg radiolabeled, single dose by oral gavage. **(3)** 1000 mg/kg radiolabeled, single dose by oral gavage. **(4)** 1000 mg/kg unlabeled, 14 days in the diet, PLUS 1000 mg/kg radiolabeled, single dose by oral gavage.	The mean radioactivity recovery 168 hours after exposure ranged between 90.6-96.7%, with the majority of the dose recovered within 48 hours in the feces (81.9-93.9%) and urine (1.26-3.07%). The maximum concentration found in the plasma of both males and females occurred approximately one hour after the administration of a single low or high dose. Between one and six hours after a single low or high dose, more kasugamycin accumulated in the kidneys, urinary bladder, and lymph nodes than in the blood, but after 168 hours, little or no kasugamycin was found in these tissues. The absorption and metabolism of kasugamycin in rats was limited (<5% dose) and was not affected by sex, dose level, or duration of dosing. Parent compound was the major component identified in the urine, feces, liver, kidney, and plasma. Minor amounts (<1% dose) of the metabolite kasuganobiosamine were identified in urine, liver, kidney, and plasma, but none was detected in the feces. Elimination occurred primarily in the feces (87.7-94.5%); however, kasugamycin was not excreted in the bile (enterohepatic circulation did not occur).

Toxicological Endpoints

TABLE 5 Summary of Toxicological Doses and Endpoints for Kasugamycin Used in the Human Health Risk Assessments.			
Exposure Scenario	**Dose Used in Risk Assessment and UF [1]**	**Special FQPA SF [2] and Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Acute Dietary (females 13 to 49 years of age)	None	None	**Not selected** No appropriate dose and endpoint could be identified for these population groups.
Acute Dietary (general population including infants and children)	None	None	**Not selected** No appropriate dose and endpoint could be identified for these population groups.
Chronic Dietary (all populations)	NOAEL = 11.3 mg/kg/day UF = 100 **Chronic RfD** = 0.113 mg/kg/day	FQPA SF =1 **cPAD** = chronic RfD / FQPA SF = 0.113 mg/kg/day	**Combined chronic toxicity/oncogenicity study in rats** LOAEL = 116 mg/kg/day based on increased testicular softening and atrophy.
Cancer (oral, dermal, inhalation)	**Classification:** No oncogenic potential was noted in the mouse oncogenicity or in the rat combined chronic/carcinogenicity studies; additionally, no mutagenic potential was noted in any of the five mutagenicity studies. Classification of kasugamycin is "not likely to be carcinogenic to humans".		

1. UF = Uncertainty Factor.
2. FQPA SF = special FQPA Safety Factor.
3. NOAEL = No Observed Adverse Effect Level.
4. RfD = Reference Dose.
5. PAD = Population-Adjusted Dose (a = acute, c = chronic).
6. LOAEL = Lowest Observed Adverse Effect Level.

Food Quality Protection Act Considerations

FQPA Safety Factor:

Based on the hazard and exposure data, the Agency has reduced the special FQPA SF to 1X because there are no/low concerns and no residual uncertainties with regard to pre- and/or post-natal toxicity. This recommendation is based on the following:

(1) there are no data gaps for the assessment of the effects of kasugamycin following *in utero* and/or post-natal exposure; a developmental neurotoxicity study is not required;

(2) there is no indication of increased quantitative or qualitative susceptibility of rats or rabbits to *in utero* and/or post-natal exposure to kasugamycin;

(3) the acute and chronic dietary food exposure assessments utilize proposed tolerance level or higher residues and 100% crop treated information for all commodities; and

(4) there are no existing or proposed residential uses for kasugamycin at this time.

Exposure Assessment

The sole anticipated exposure route to Kasugamycin for the US population is via dietary (food only) exposure. There are no proposed US registrations for kasugamycin and there is no expectation that kasugamycin residues would occur in surface or ground water sources of drinking water. Therefore, no aggregate nor occupational exposure is expected. A summary of exposure assessments follows:

- An acute exposure assessment was not preformed as no appropriate dose and endpoint was selected for any population subgroup.
- A chronic exposure assessment was conducted assuming tolerance level residues and 100% crop treated. The chronic population adjusted dose for all population subgroups including the general U.S. population was < 1%.
- A cancer exposure assessment was not preformed as kasugamycin was classified as "not likely to be carcinogenic to humans".

Public Health Summary

Kasugamycin is a new active ingredient and, as such, no public health data are currently available. Kasugamycin operates via a mode of action different from that of the other aminoglycoside antibiotics such as streptomycin. Because kasugamycin is active only against phytopathogenic fungi and bacteria, it has never been employed as a human or veterinary-use antibiotic.

The Agency is aware that FDA and CDC have concerns regarding the potential for antibiotics to induce bacterial resistance arising from their use as pesticides. The Agency has met with these other agencies recently to discuss resistance issues and an ongoing dialogue is anticipated. The Agency's level of concern is low regarding development of resistance (associated with kasugamycin's use as a fungicide) arising from tolerances for kasugamycin on imported fruiting vegetables because:

- proposed use rates for kasugamycin are low, and residues following its application are either very low or non-detectable
- the proposed uses are only on imported fruiting vegetables, with no proposed domestic uses, and
- there are no human or veterinary uses of kasugamycin as an antibiotic.

SUMMARY OF DATA GAPS

860.1340 Residue Analytical Method: The analytical enforcement method uses ion exchange resins for clean up and reverse-phase ion-pairing liquid chromatography with ultra-violet detection (HPLC/UV). This method was validated by an independent laboratory. The Agency's laboratory also conducted a laboratory trial of this method and has suggested that the substitution of solid-phase extraction for the ion-exchange process could possibly improve the method. The Agency will request that changes be made to the method.

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DISCLAIMER: The information in this Pesticide Fact Sheet is for information only and is not to be used to satisfy data requirements for pesticide registration. The information is believed to be accurate as of the date on the document.

APPENDIX I:

GLOSSARY OF TERMS AND ABBREVIATIONS

ADNT	**Acute delayed neurotoxicity**
a.i.	**Active Ingredient**
aPAD	**Acute Population Adjusted Dose**
ARI	**Aggregate Risk Index**
BCF	**Bioconcentration Factor**
CAS	**Chemical Abstracts Service**
ChE	**Cholinesterase**
ChEI	**Cholinesterase inhibition**
cPAD	**Chronic Population Adjusted Dose**
%CT	**Percent crop treated**
DAT	**Days after treatment**
DEEM-FCID	**Dietary Exposure Evaluation Model - Food Consumption Intake Database**
DNA	**Deoxyribonucleic acid**
DNT	**Developmental neurotoxicity**
DIT	**Developmental immunotoxicity**
DWLOC	**Drinking Water Level of Comparison.**
EC	**Emulsifiable Concentrate Formulation**
EEC	**Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.**
EPA	**U.S. Environmental Protection Agency**
FQPA	**Food Quality Protection Act**
GLC	**Gas Liquid Chromatography**
GLN	**Guideline Number**
LC_{50}	**Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/l, mg/kg or ppm.**
LD_{50}	**Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.**
LOAEL	**Lowest Observed Adverse Effect Level**
LOAEC	**Lowest Observed Adverse Effect Concentration**
LOC	**Level of Concern**
LOD	**Limit of Detection**
LOQ	**Limit of quantitation**
mg/kg/day	**Milligram Per Kilogram Per Day**
mg/L	**Milligrams Per Liter**
MOE	**Margin of Exposure**
MRID	**Master Record Identification (number), EPA's system of recording and tracking studies submitted**
MTD	**Maximum tolerated dose**
NA	**Not Applicable**

NOEC No Observable Effect Concentration
NOEL No Observed Effect Level
NOAEL No Observed Adverse Effect Level
NOAEC No Observed Adverse Effect Concentration
NPDES National Pollutant Discharge Elimination System
OP Organophosphate
OPP EPA Office of Pesticide Programs
OPPTS EPA Office of Prevention, Pesticides and Toxic Substances
PAD Population Adjusted Dose
PAG Pesticide Assessment Guideline
PAM Pesticide Analytical Method
PHED Pesticide Handler's Exposure Data
PHI Preharvest Interval
ppb Parts Per Billion
PPE Personal Protective Equipment
ppm Parts Per Million
PRZM/EXAMS Tier II Surface Water Computer Model
RAC Raw Agriculture Commodity
RBC Red Blood Cell
RED Reregistration Eligibility Decision
REI Restricted Entry Interval
RfD Reference Dose
SCI-GROW Tier I Ground Water Computer Model
SF Safety Factor
TGAI Technical Grade Active Ingredient
UF Uncertainty Factor
μg micrograms
μg/L Micrograms Per Liter
μL/g Microliter per gram
USDA United States Department of Agriculture
WPS Worker Protection Standard

Appendix II

Citations Considered to be Part of the Data Base Supporting the Registration of Kasugamycin

45910000	Hokko Chemical Industry Co., Ltd. (2003) Submission of Product Chemistry, Residue, Fate and Toxicity Data in Support of the Petition for Tolerance of Kasugamycin on Fruiting Vegetables, Crop Group 8, Except Cucurbits. Transmittal of 28 Studies.
45910001	Bujor, D. (2003) Kasugamycin--Food Quality Protection Act Supplemental Information to Support Use on Fruiting Vegetables, Crop Group 8: Lab Project Number: KAS-FQPA-01. Unpublished study prepared by Arvesta Corporation. 45 p.
45910002	Pesselman, R. (1993) Analysis of Product Ingredients in Kasugamycin: Lab Project Number: TMN-073: HWI 6293-115. Unpublished study prepared by Hazleton Wisconsin, Inc. 102 p.
45910003	Curry, K.; Brookman, D.; Bujor, D. (2003) Kasugamycin Technical: Product Properties--Group A: Product Identity and Composition, Materials Used to Produce the Product, Description of Production, Discussion of Formation of Impurities, Preliminary Analysis: Lab Project Number: TMN-074. Unpublished study prepared by Technology Sciences Group, Inc. 66 p. {OPPTS 830.1550, 830.1600, 830.1620, 830.1670, 830.1700, 830.1650}
45910004	Brookman, D.; Curry, K. (2003) Group B: Product Properties--Kasugamycin Technical: Color, Physical State, Odor, Melting Point, Boiling Point, pH, Density, Dissociation Constant, Octanol/Water Partition Coefficient, Water Solubility, Vapor Pressure, Stability to Normal and Elevated Temperature, Metals, and Metal Ions: Lab Project Number: TMN-073A. Unpublished study prepared by Technology Sciences Group, Inc. 7 p. {OPPTS 830.6302, 830.6303, 830.6304, 830.6313, 830.7000, 830.7200, 830.7300, 830.7370, 830.7550, 830.7560, 830.7570, 830.7840, 830.7860, 830.7950}
45910005	Pesselman, R. (1993) Series 63 Product Chemistry Determination of Kasugamycin: Color, Physical State, Odor, Melting Point, Density, Solubility, Vapor Pressure, Dissociation Constant, Octanol/Water Partition Coefficient, pH, Stability: Lab Project Number: TMN-0072: HWI 6293-16. Unpublished study prepared by Hazleton Wisconsin, Inc. 110 p.
45910006	Cooke, J. (2002) Metabolic Fate and Distribution of (Carbon 14)-Kasugamycin in Tomato: Lab Project Number: TMN-0063: 1442/12/D2149. Unpublished study prepared by Covance Laboratories, Ltd. 98 p. {OPPTS 860.1300}
45910007	Faltynski, K. (2002) Independent Laboratory Validation (ILV) of Morse Laboratory's Method for the Analysis of Kasumin (TM-416) in Crop: Lab

Project Number: TMN-0082A: 01-0047: METH-146. Unpublished study prepared by En-Cas Analytical Laboratories. 88 p. {OPPTS 860.1340}

45910008 Westberg, G. (2003) Validation of the Analytical Method for the Determination of Kasugamycin in Tomatoes, Potatoes and Peppers: Lab Project Number: TMN-0081 A: MLIR-03-01: METH-146. Unpublished study prepared by Morse Laboratories, Inc. 61 p.

45910009 Fomenko, J. (2002) Evaluation of TM-416 through the FDA Multiresidue Methods: Lab Project Number: TMN-0081: A055.002. Unpublished study prepared by Maxim Technologies, Inc. 43 p. {OPPTS 860.1360}

45910010 Carringer, S. (2002) Magnitude of the Residue of Kasugamycin in Pepper Raw Agricultural Commodities: Lab Project Number: TMN-0092: TCI-01-012: ML01-0989-TOM. Unpublished study prepared by Morse Laboratories, Inc. 209 p. {OPPTS 860.1500}

45910011 Carringer, S. (2002) Magnitude of the Residue of Kasugamycin in Tomato Raw Agricultural Commodities: Lab Project Number: TMN-0099B: TCI-01-011: ML01-0987-TOM. Unpublished study prepared by Morse Laboratories, Inc. 283 p. {OPPTS 860.1500}

45910012 Glaza, S. (1992) Acute Oral Toxicity Study of Kasugamycin Hydrochloride Technical in Rats: Lab Project Number: HWI 20504630: TMN-0113: TP3013. Unpublished study prepared by Hazleton Wisconsin, Inc. 24 p.

45910013 Cuthbert, J.; Jackson, D. (1992) Kasumin Liquid: Acute Oral Toxicity (Limit) Test in Mice: Lab Project Number: TMN-0106: 553046-9018. Unpublished study prepared by Inveresk Research International. 90 p.

45910014 Cuthbert, J.; Jackson, D. (1992) Kasumin Liquid: Acute Oral Toxicity (Limit) Test in Rats: Lab Project Number: TMN-0107: 553046-9017. Unpublished study prepared by Inveresk Research International. 16 p.

45910015 Cuthbert, J.; Jackson, D.; Pallas, E. (1992) Kasumin Liquid: Primary Eye Irritation Test in Rabbits: Lab Project Number: TMN-0110: 553046-9021. Unpublished study prepared by Inveresk Research International. 18 p.

45910016 Cuthbert, J.; Jackson, D. (1992) Kasumin Liquid: Buehler Sensitization Test in Guinea Pigs: Lab Project Number: TMN-0111: 553046-9022. Unpublished study prepared by Inveresk Research International. 24 p.

45910017 Cuthbert, J.; Jackson, D.; Pallas, E. (1992) Kasumin Liquid: Primary Skin Irritation Test in Rabbits: Lab Project Number: TMN-0109: 553046-9020. Unpublished study prepared by Inveresk Research International. 16 p.

45910018 Sheperd, N. (2001) Kasumin 2 L: Single Exposure (Nose Only) Toxicity Study in the Rat: Lab Project Number: TMN-0112: 1442/8-D6145: 1442/8. Unpublished study prepared by Covance Laboratories. 84 p. {OPPTS

870.1300}

45910019 Holmes, P. (1990) Kasugamycin: Toxicity Study by Dietary Administration to CD-1 Mice for 13 Weeks: Lab Project Number: TMN-0156: LSR 90/0345. Unpublished study prepared by Life Science Research Limited. 301 p. {OPPTS 870.3100}

45910020 Nakashima, N. (1991) Kasugamycin: 13-Week Oral Subchronic Toxicity Study in Rats: Lab Project Number: TMN-0154: IET 89-0083. Unpublished study prepared by The Institute of Environmental Toxicology. 228 p.

45910021 Fujii, S. (1990) Teratogenicity Study in Rats with Kasugamycin: Preliminary Study: Lab Project Number: TMN-0135: IET 89-0084: KK 02-12. Unpublished study prepared by The Institute of Environmental Toxicology. 74 p.

45910022 Fujii, S. (1991) Teratogenicity Study in Rats with Kasugamycin: Lab Project Number: TMN-0136: IET 89-0085. Unpublished study prepared by The Institute of Environmental Toxicology. 72 p.

45910023 Henwood, S. (1993) Two-Generation Reproduction Study with Kasugamycin in Rats: Lab Project Number: TMN-0126: HWI 6434-102: TP2025. Unpublished study prepared by Hazleton Wisconsin, Inc. 1349 p.

45910024 Kitazawa, T. (1987) Kasugamycin: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats: Lab Project Number: TMN-0120: ID-09-1987. Unpublished study prepared by The Institute of Environmental Toxicology. 880 p.

45910025 Ivett, J. (1985) Mutagenicity Evaluation of Kasugamycin Technical (Purity 67.1% Lot. No. KP-570) in an in Vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells: Lab Project Number: TMN-144: 20990: 437. Unpublished study prepared by Litton Bionetics, Inc. 21 p.

45910026 Young, R. (1985) Evaluation of Kasugamycin (Lot. No. KP-570) in the V79/HGPRT Forward Mutation Assay: Lab Project Number: TMN-0142: 22207: 436. Unpublished study prepared by Litton Bionetics, Inc. 26 p.

45910027 Seeberg, A. (1985) Kasugamycin: Unscheduled DNA Synthesis in Human Cells Cell Line: Hela S3: Lab Project Number: TMN-0145: 161001-M-01885: 161-001. Unpublished study prepared by Life Science Research. 48 p.

45910028 Shirasu, Y.; Moriya, M.; Watanabe, Y. (1976) Mutagenicity Testing on Kasugamycin-HCL in Microbial Systems: Lab Project Number: TMN-0143: IET-11-15-1976. Unpublished study prepared by The Institute of Environmental Toxicology. 12 p.

46030300 Arvesta Corporation (2003) Submission of Toxicity Data in Support of the Petition for Tolerance of Kasugamycin on Fruiting Vegetables, Crop Group 8.

Transmittal of 6 Studies.

46030301 Cuthbert, J.; Jackson, D. (1992) Kasumin Liquid: Acute Dermal Toxicity (Limit) Test in Rats. Project Number: TMN/0108, 553046/9019, 553046. Unpublished study prepared by Inveresk Research International. 18 p.

46030302 Thomford, P. (1993) 13-Week Dietary Toxicity Study with Kasugamycin in Dogs. Project Number: TMN/0155, HWI/6434/101. Unpublished study prepared by Hazleton Wisconsin, Inc. 399 p.

46030303 Ross, F. (1986) Kasugamycin: Teratology Study in the Rabbit. Project Number: TMN/0134, 86/HKC004/114. Unpublished study prepared by Life Science Research. 63 p.

46030304 Holmes, P. (1992) Kasugamycin: Oncogenicity Study by Dietary Administration to CD-1 Mice for 78 Weeks: (Final Report). Project Number: TMN/0122, 91/HKC006/1010. Unpublished study prepared by Life Science Research. 673 p.

46030305 Hodson-Walker, G. (1985) Kasugamycin: Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test: (Final Report). Project Number: TMN/0146, 85/HKC001/207. Unpublished study prepared by Life Science Research. 52 p.

46030306 Cheng, T. (1998) Metabolism of (Carbon 14)-Kasugamycin in Rats. Project Number: TMN/0059, 6434/110. Unpublished study prepared by: Covance Laboratories, Inc. 512 p.

46185900 Hokko Chemical Industry Co., Ltd. (2004) Submission of Toxicity Data in Support of the Petition for Tolerance of Kasugamycin. Transmittal of 1 Study.

46185901 Albertsen, J. (2003) 52-Week Dietary Toxicity Study with Kasugamycin in Dogs: (Final Report). Project Number: 6434/117. Unpublished study prepared by Covance Laboratories, Inc. 571 p.

46428700 Hokko Chemical Industry Co., Ltd. (2004) Submmission of Toxicity Data in Support of the Petition for Tolerance of Kasugamycin. Transmittal of 1 Study.

46428701 Tesh, J.; Ross, F.; Wright, P. (1986) Kasugamycin: Preliminary Teratology Study in the Rabbit. Project Number: 85/HKC003/783. Unpublished study prepared by Life Science Research. 47 p.

46444900 Hokko Chemical Industry Corp. (2005) Submission of Toxicity Data in Support of the Petition for Tolerance of Kasugamycin. Transmittal of 1 Study.

46444901 Burin, G. (2005) Study Waiver Request for Subchronic Dermal, Acute Neurotoxicity and Subchronic Neurotoxicity Studies of Kasugamycin. Unpublished study prepared by Technology Sciences Group, Inc. 5 p.

46448300	Hokko Chemical Industry Co., Ltd. (2005) Submission of Toxicity Data in Support of the Petition for Tolerance of Kasugamycin. Transmittal of 1 Study.
46448301	Holmes, P. (1992) Kasugamycin: Oncogenicity Study by Dietary Administration to CD-1 Mice for 78 Weeks. Project Number: TMN/0122, 91/HKC006/1010. Unpublished study prepared by Life Science Research. 1426 p.
46485500	Hokko Chemical Industry Co., Ltd. (2005) Submission of Environmental Fate Data in Support of the Petition for Tolerance of Kasugamycin for Use on Fruiting Vegetables. Transmittal of 1 Study.
46485501	Swales, S. (2003) (Carbon 14) Kasugamycin: Hydrolytic Stability. Project Number: 1442/21. Unpublished study prepared by Covance Laboratories, Ltd. 123 p.

11. Indian Tribal Governments

This proposed rule does not have tribal implications under Executive Order 13175, Consultation and Coordination with Indian Tribal Governments, because it would not have a substantial direct effect on one or more Indian tribes, on the relationship between the Federal Government and Indian tribes, or on the distribution of power and responsibilities between the Federal Government and Indian tribes.

12. Energy Effects

This proposed rule is not a ''significant energy action'' under Executive Order 13211, Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use.

13. Technical Standards

This proposed rule does not use technical standards. Therefore, we did not consider the use of voluntary consensus standards.

14. Environment

We have analyzed this proposed rule under Department of Homeland Security Management Directive 023–01 and Commandant Instruction M16475.lD, which guide the Coast Guard in complying with the National Environmental Policy Act of 1969 (NEPA) (42 U.S.C. 4321–4370f), and have made a preliminary determination that this action is one of a category of actions that do not individually or cumulatively have a significant effect on the human environment. This proposed rule establishes a temporary safety zone to protect the public from fireworks fallout. This rule is categorically excluded from further review under paragraph 34(g) of Figure 2–1 of the Commandant Instruction. A preliminary environmental analysis checklist supporting this determination and a Categorical Exclusion Determination are available in the docket where indicated under **ADDRESSES**. We seek any comments or information that may lead to the discovery of a significant environmental impact from this proposed rule.

List of Subjects in 33 CFR Part 165

Harbors, Marine safety, Navigation (water), Reporting and recordkeeping requirements, Security measures, and Waterways.

For the reasons discussed in the preamble, the Coast Guard proposes to amend 33 CFR part 165 as follows:

PART 165—REGULATED NAVIGATION AREAS AND LIMITED ACCESS AREAS

■ 1. The authority citation for part 165 continues to read as follows:

Authority: 33 U.S.C. 1231; 46 U.S.C. Chapter 701, 3306, 3703; 50 U.S.C. 191, 195; 33 CFR 1.05–1, 6.04–1, 6.04–6, 160.5; Pub. L. 107–295, 116 Stat. 2064; Department of Homeland Security Delegation No. 0170.1.

■ 2. Add temporary § 165.T05–0723 to read as follows:

§ 165.T05–0723 Safety Zone, Shallowbag Bay; Manteo, NC.

(a) *Definitions.* For the purposes of this section, Captain of the Port means the Commander, Sector North Carolina. *Representative* means any Coast Guard commissioned, warrant, or petty officer who has been authorized to act on the behalf of the Captain of the Port.

(b) *Location.* The following area is a safety zone: This safety zone will encompass all waters on Shallowbag Bay within a 200 yard radius of a barge anchor in position 35°54′31″ N, longitude 075°39′42″ W. All geographic coordinates are North American Datum 1983 (NAD 83).

(c) *Regulations.* (1) The general regulations contained in § 165.23 of this part apply to the area described in paragraph (b) of this section.

(2) Persons or vessels requiring entry into or passage through any portion of the safety zone must first request authorization from the Captain of the Port, or a designated representative, unless the Captain of the Port previously announced via Marine Safety Radio Broadcast on VHF Marine Band Radio channel 22 (157.1 MHz) that this regulation will not be enforced in that portion of the safety zone. The Captain of the Port can be contacted at telephone number (910) 343–3882 or by radio on VHF Marine Band Radio, channels 13 and 16.

(d) *Enforcement.* The U.S. Coast Guard may be assisted in the patrol and enforcement of the zone by Federal, State, and local agencies.

(e) *Enforcement period.* This section will be enforced from 8 p.m. to 10 p.m. on September 26, 2014 unless cancelled earlier by the Captain of the Port.

Dated: August 14, 2014.

S. R. Murtagh,

Captain, U.S. Coast Guard, Captain of the Port North Carolina.

[FR Doc. 2014–20675 Filed 8–28–14; 8:45 am]

BILLING CODE 9110–04–P

ENVIRONMENTAL PROTECTION AGENCY

Appendix D

40 CFR Part 180

[EPA–HQ–OPP–2010–0297; FRL–9911–57]

Kasugamycin; Pesticide Tolerances

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This regulation establishes a tolerance for residues of kasugamycin in or on fruit, pome. Arysta LifeScience North America, LLC (Arysta LifeScience), requested a number of tolerances under the Federal Food, Drug, and Cosmetic Act (FFDCA) which are addressed in this document.

DATES: This regulation is effective August 29, 2014. Objections and requests for hearings must be received on or before October 28, 2014, and must be filed in accordance with the instructions provided in 40 CFR part 178 (see also Unit I.C. of the **SUPPLEMENTARY INFORMATION**).

ADDRESSES: The docket for this action, identified by docket identification (ID) number EPA–HQ–OPP–2010–0297, is available at *http://www.regulations.gov* or at the Office of Pesticide Programs Regulatory Public Docket (OPP Docket) in the Environmental Protection Agency Docket Center (EPA/DC), West William Jefferson Clinton Bldg., Rm. 3334, 1301 Constitution Ave. NW., Washington, DC 20460–0001. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the OPP Docket is (703) 305–5805. Please review the visitor instructions and additional information about the docket available at *http://www.epa.gov/dockets.*

FOR FURTHER INFORMATION CONTACT: Lois Rossi, Registration Division (7505P), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001; telephone number: (703) 305–7090; email address: *RDFRNotices@epa.gov.*

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this action apply to me?

You may be potentially affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. The following list of North American Industrial Classification System (NAICS) codes is not intended to be exhaustive, but rather provides a guide to help readers

determine whether this document applies to them. Potentially affected entities may include:

- Crop production (NAICS code 111).
- Animal production (NAICS code 112).
- Food manufacturing (NAICS code 311).
- Pesticide manufacturing (NAICS code 32532).

B. How can I get electronic access to other related information?

You may access a frequently updated electronic version of EPA's tolerance regulations at 40 CFR part 180 through the Government Printing Office's e-CFR site at *http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?&c=ecfr&tpl=/ecfrbrowse/Title40/40tab_02.tpl.* To access the OCSPP test guidelines referenced in this document electronically, please go to *http://www.epa.gov/ocspp* and select ''Test Methods and Guidelines.''

C. How can I file an objection or hearing request?

Under FFDCA section 408(g), 21 U.S.C. 346a, any person may file an objection to any aspect of this regulation and may also request a hearing on those objections. You must file your objection or request a hearing on this regulation in accordance with the instructions provided in 40 CFR part 178. To ensure proper receipt by EPA, you must identify docket ID number EPA–HQ–OPP–2010–0297 in the subject line on the first page of your submission. All objections and requests for a hearing must be in writing, and must be received by the Hearing Clerk on or before October 28, 2014. Addresses for mail and hand delivery of objections and hearing requests are provided in 40 CFR 178.25(b).

In addition to filing an objection or hearing request with the Hearing Clerk as described in 40 CFR part 178, please submit a copy of the filing (excluding any Confidential Business Information (CBI)) for inclusion in the public docket. Information not marked confidential pursuant to 40 CFR part 2 may be disclosed publicly by EPA without prior notice. Submit the non-CBI copy of your objection or hearing request, identified by docket ID number EPA–HQ–OPP–2010–0297, by one of the following methods:

- *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be CBI or other information whose disclosure is restricted by statute.
- *Mail:* OPP Docket, Environmental Protection Agency Docket Center (EPA/DC), (28221T), 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001.
- *Hand Delivery:* To make special arrangements for hand delivery or delivery of boxed information, please follow the instructions at *http://www.epa.gov/dockets/contacts.html.*

Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at *http://www.epa.gov/dockets.*

II. Summary of Petitioned-For Tolerance

In the **Federal Register** of May 19, 2010 (75 FR 28009) (FRL–8823–2), EPA issued a document pursuant to FFDCA section 408(d)(3), 21 U.S.C. 346a(d)(3), announcing the filing of a pesticide petition (PP 0F7689) by Arysta LifeScience North America, LLC, 15401 Weston Parkway, Cary, NC 27513. The petition requested that 40 CFR 180.614 be amended by establishing tolerances for residues of the fungicide kasugamycin, in or on fruiting vegetables (crop group 8) at 0.15 parts per million (ppm), pome fruit (crop group 11) at 0.25 ppm, and walnuts at 0.04 ppm. That document referenced a summary of the petition prepared by Arysta LifeScience, the registrant, which is available in the docket, *http://www.regulations.gov*. There were no comments received in response to the notice of filing.

Based upon review of the data supporting the petition, EPA has modified the proposed tolerance levels and the crops for which tolerances will be established. The reasons for these changes are explained in Unit IV.C. The tolerance in imported fruiting vegetables, crop group 8 is not being removed or revised at this time. This regulation additionally deletes the time-limited tolerance for apple, as the tolerance will be superseded by permanent tolerances in the various pome fruits.

III. Aggregate Risk Assessment and Determination of Safety

Section 408(b)(2)(A)(i) of FFDCA allows EPA to establish a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the tolerance is ''safe.'' Section 408(b)(2)(A)(ii) of FFDCA defines ''safe'' to mean that ''there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information.'' This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Section 408(b)(2)(C) of FFDCA requires EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to ''ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue. . . .''

Consistent with FFDCA section 408(b)(2)(D), and the factors specified in FFDCA section 408(b)(2)(D), EPA has reviewed the available scientific data and other relevant information in support of this action. EPA has sufficient data to assess the hazards of and to make a determination on aggregate exposure for kasugamycin on pome commodities, including exposure resulting from the tolerances established by this action. EPA's assessment of exposures and risks associated with kasugamycin follows.

A. Toxicological Profile

EPA has evaluated the available toxicity data and considered its validity, completeness, and reliability as well as the relationship of the results of the studies to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

Kasugamycin is a member of the aminoglycoside family of antibiotics, which also includes streptomycin and gentamicin. These agents inhibit bacterial protein synthesis by binding to the 30S subunit of the bacterial ribosome. Their penetration through the cell membrane of the bacterium depends partly on oxygen-dependent active transport by a polyamine carrier system that seems to be absent in mammalian systems.

Kasugamycin exhibits low acute toxicity, being only a mild dermal and ocular irritant. The major effects observed across species in multiple-dose studies were decreased body weights and body weight gains. The primary target organs identified for kasugamycin were the testes and kidney in the rat and mouse. However, these effects were only seen at higher dose levels, generally at the highest dose tested (HDT). In the combined chronic toxicity/carcinogenicity study in rats, the basis for the lowest-observed-adverse-effect level (LOAEL) was an increased incidence and severity of testicular tubular atrophy, observed during the histopathologic examinations at the end of the 2-year dosing period, as well as at 6 months, and 1 year. Testicular degeneration and atrophy

were observed in adult F1 males in the rat reproductive toxicity study at the highest dose. Testicular tubular dilatation and degeneration were observed in the mouse subchronic study, but at a dose that exceeded the limit dose; the mouse carcinogenicity study tested at much lower doses, and these effects were not observed. In the dog chronic toxicity study, testicular inflammation was reported at the high dose, but was not accompanied by atrophic or degenerative changes, and was not considered a treatment-related adverse effect.

Kidney toxicity is often associated with exposure to aminoglycoside antibiotics, and the metabolism study indicated higher levels of radioactivity in the kidneys than other tissues. In male F1 rats in the reproductive toxicity study, dilatation of the kidney, and an increased incidence of chronic progressive nephropathy were observed. In the subchronic rat study, an increased incidence of eosinophilic bodies (graded slight for severity) in the renal proximal tubular cells was reported in males at several dose levels. These effects were considered treatment related, but not adverse due to their low severity grade, and lack of associated findings. However, in female rats, increased epithelial cells in the urinary sediment, along with decreased urine pH (decreased pH was also seen in males), were observed at the high dose, and considered evidence of possible kidney toxicity. Lipofuscin deposition (slight) was observed in the rat combined chronic toxicity/carcinogenicity study, but was not considered adverse due to the lack of other related findings; this study tested up to the no-observed-adverse-effect level (NOAEL) of the subchronic study. In the mouse, following subchronic exposure, minimal to severe basophilia/hyperplasia in the renal *pars recta* in females was observed. No renal effects were reported in the mouse carcinogenicity study at lower doses, or in the dog subchronic or chronic studies.

There was no evidence that exposure to kasugamycin results in neurotoxicity, and a developmental neurotoxicity (DNT) study is not required. Also, there was no evidence of immune system effects based on the review of a submitted immunotoxicity study. Although a 28-day rat inhalation toxicity study was not submitted, EPA has determined that it is not required based on available hazard and exposure information.

The database is complete with respect to pre- and postnatal toxicity, and shows no evidence of increased qualitative or quantitative susceptibility in the offspring, or in the developing fetus. There was no evidence of carcinogenicity in male and female mice, nor in male and female rats at doses that were adequate to assess the carcinogenic potential of kasugamycin. There was no evidence of mutagenicity. Based on the overall weight of the evidence, kasugamycin is classified as ''not likely to be carcinogenic to humans.''

Although antimicrobial drug residues present in or on food may cause adverse effects on the ecology of the intestinal microflora of consumers, the Agency does not believe this is a concern for kasugamycin because of the use pattern (application occurring prior to fruit development) and low residue detection in field trials.

Specific information on the studies received and the nature of the adverse effects caused by kasugamycin as well as the NOAEL and the LOAEL from the toxicity studies can be found at *http://www.regulations.gov* in document ''Kasugamycin. Human Health Risk Assessment for the Proposed Use of the Fungicide on Fruiting Vegetables, Pome Fruits, and Walnuts'' at pp. 15–21 in docket ID number EPA–HQ–OPP–2010–0297.

B. Toxicological Points of Departure/Levels of Concern

Once a pesticide's toxicological profile is determined, EPA identifies toxicological points of departure (POD) and levels of concern (LOC) to use in evaluating the risk posed by human exposure to the pesticide. For hazards that have a threshold below which there is no appreciable risk, the toxicological POD is used as the basis for derivation of reference values for risk assessment. PODs are developed based on a careful analysis of the doses in each toxicological study to determine the dose at which NOAEL and the LOAEL are identified. Uncertainty/safety factors are used in conjunction with the POD to calculate a safe exposure level—generally referred to as a population-adjusted dose (PAD) or a reference dose (RfD)—and a safe margin of exposure (MOE). For non-threshold risks, the Agency assumes that any amount of exposure will lead to some degree of risk. Thus, the Agency estimates risk in terms of the probability of an occurrence of the adverse effect expected in a lifetime. For more information on the general principles EPA uses in risk characterization and a complete description of the risk assessment process, see *http://www.epa.gov/pesticides/factsheets/riskassess.htm.* A summary of the toxicological endpoints for kasugamycin used for human risk assessment is shown in Table 1 of this unit.

TABLE 1—SUMMARY OF TOXICOLOGICAL DOSES AND ENDPOINTS FOR KASUGAMYCIN RELEVANT TO FFDCA ANALYSIS

Exposure scenario	Point of departure	Uncertainty and FQPA SF	RfD, PAD, LOC for risk assessment	Study and toxicological effects
Acute dietary (all populations)	An appropriate dose and endpoint for this risk assessment scenario was not identified, based on a lack of single-dose effects in the database.			
Chronic dietary (all populations including infants and children, and females age 13 to 49).	NOAEL = 11 mg/kg/day.	UF_A = 10X UF_H = 10X FQPA SF = 1X	Chronic RfD = 0.11 mg/kg/day. cPAD = 0.11 mg/kg/day.	Combined chronic toxicity/carcinogenicity study in the rat. LOAEL = 116 mg/kg/day, based on testicular atrophy and softening.
Cancer (oral, dermal, inhalation)	Classification: ''Not likely to be carcinogenic to humans.''			

Point of Departure = a data point or estimated point derived from observed dose-response data, which is used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). FQPA SF = Food Quality Protection Act Safety Factor. NOAEL = no-observed-adverse-effect level. LOAEL = lowest-observed-adverse-effect level. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. LOC = level of concern.

C. Exposure Assessment

1. *Dietary exposure from food and feed uses.* In evaluating dietary exposure to kasugamycin, EPA considered exposure under the petitioned-for tolerances as well as all existing kasugamycin tolerances in 40 CFR 180.614. EPA assessed dietary exposures from kasugamycin in food as follows:

i. *Acute exposure.* Quantitative acute dietary exposure and risk assessments are performed for a food-use pesticide, if a toxicological study has indicated the possibility of an effect of concern occurring as a result of a 1-day or single exposure. No such effects were identified in the toxicological studies for kasugamycin; therefore, a quantitative acute dietary exposure assessment is unnecessary.

ii. *Chronic exposure.* In conducting the chronic dietary exposure assessment, EPA used the food consumption data from the United States Department of Agriculture (USDA) 1994–1996 and the 1998 Nationwide Continuing Surveys of Food Intake by Individuals (CSFII). An unrefined chronic aggregate dietary (food and drinking water) exposure and risk assessment was conducted using the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database (DEEM–FCID™), Version 2.03. The residue inputs into the dietary model were the recommended tolerance level residues and default processing factors were used, with the exception of the apple juice processing factor, for which the 1.5X data-derived processing factor was used. EPA assumed 100% crop treated (PCT) for all proposed uses.

iii. *Cancer.* Based on the data summarized in Unit III.A., EPA has concluded that kasugamycin does not pose a cancer risk to humans. Therefore, a quantitative dietary exposure assessment for the purpose of assessing cancer risk is unnecessary.

iv. *Anticipated residue and PCT information.* EPA did not use anticipated residue and/or PCT information in the dietary assessment for kasugamycin. Tolerance level residues and/or 100 PCT were assumed for all food commodities.

2. *Dietary exposure from drinking water.* The Agency used screening level water exposure models in the dietary exposure analysis and risk assessment for kasugamycin in drinking water. These simulation models take into account data on the physical, chemical, and fate/transport characteristics of kasugamycin. Further information regarding EPA drinking water models used in pesticide exposure assessment can be found at *http://www.epa.gov/oppefed1/models/water/index.htm.*

Based on the Pesticide Root Zone Model/Exposure Analysis Modeling System (PRZM/EXAMS) the estimated drinking water concentrations (EDWCs) of kasugamycin for chronic exposures for non-cancer assessments are estimated to be 0.001178 ppm for surface water. EDWCs of kasugamycin for ground water were estimated to be 0.000116 ppm via the Screening Concentration in Ground Water (SCI–GROW) system. Modeled estimates of drinking water concentrations were directly entered into the dietary exposure model. For chronic dietary risk assessment, the water concentration of value 0.001178 ppm was used to assess the contribution to drinking water.

3. *From non-dietary exposure.* The term ''residential exposure'' is used in this document to refer to non-occupational, non-dietary exposure (e.g., for lawn and garden pest control, indoor pest control, termiticides, and flea and tick control on pets).

Kasugamycin is not registered for any specific use patterns that would result in residential exposure.

4. *Cumulative effects from substances with a common mechanism of toxicity.* Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider ''available information'' concerning the cumulative effects of a particular pesticide's residues and ''other substances that have a common mechanism of toxicity.'' EPA has not found kasugamycin to share a common mechanism of toxicity with any other substances, and kasugamycin does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has assumed that kasugamycin does not have a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see EPA's Web site at *http://www.epa.gov/pesticides/cumulative.*

D. Safety Factor for Infants and Children

1. *In general.* Section 408(b)(2)(C) of FFDCA provides that EPA shall apply an additional tenfold (10X) margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database on toxicity and exposure unless EPA determines based on reliable data that a different margin of safety will be safe for infants and children. This additional margin of safety is commonly referred to as the Food Quality Protection Act Safety Factor (FQPA SF). In applying this provision, EPA either retains the default value of 10X, or uses a different additional safety factor when reliable data available to EPA support the choice of a different factor.

2. *Prenatal and postnatal sensitivity.* There was no evidence of increased quantitative or qualitative susceptibility in rat or rabbit developmental toxicity studies, or in the rat reproductive study. No developmental effects were seen in the rat developmental study, whereas maternal toxicity (decreased body weight gain, food consumption, and feed efficiency) was observed at the highest dose. Although no maternal or developmental toxicity was observed in the main rabbit developmental toxicity study, in the dose range-finding study, maternal weight loss, reduced food consumption during dosing, and abortions (occurring at GD 18 or later) were observed at higher doses. Fetal weight was decreased at the maternally toxic dose but, due to abortions or maternal death, was not evaluated at the higher doses. In the rat reproductive toxicity study, parental toxicity included decreased parental body weight/weight gain at the mid and high doses. No offspring toxicity was observed. Reproductive toxicity was observed only at the highest dose tested (above the parental LOAEL), with testicular atrophy, decreased fertility and fecundity in the F1 parents for both litters, and an increased pre-coital interval during the mating period for the F2b litter.

3. *Conclusion.* EPA has determined that reliable data show the safety of infants and children would be adequately protected if the FQPA SF were reduced to 1X for the following reasons:

i. The toxicity database for kasugamycin is complete, including rat acute and subchronic neurotoxicity screening studies and a mouse immunotoxicity study. Based on the lack of observed neurotoxicity, a DNT study is not required. Furthermore, a 28-day inhalation study is not required based on the available hazard and exposure information and proposed and existing uses for kasugamycin.

ii. There is no evidence of increased quantitative or qualitative pre- and/or postnatal susceptibility observed in developmental toxicity studies in the rat and rabbit, or in a 2-generation reproduction study in the rat.

iii. The exposure assessment for food and drinking water will not underestimate potential dietary exposure to kasugamycin. There are no proposed or existing residential uses for kasugamycin.

E. Aggregate Risks and Determination of Safety

EPA determines whether acute and chronic dietary pesticide exposures are safe by comparing aggregate exposure estimates to the acute PAD (aPAD) and chronic PAD (cPAD). For linear cancer risks, EPA calculates the lifetime probability of acquiring cancer given the estimated aggregate exposure. Short-, intermediate-, and chronic-term risks are evaluated by comparing the estimated aggregate food, water, and residential exposure to the appropriate PODs to ensure that an adequate MOE exists.

1. *Acute risk.* An acute aggregate risk assessment takes into account acute exposure estimates from dietary consumption of food and drinking water. No adverse effect resulting from a single oral exposure was identified and no acute dietary endpoint was selected. Therefore, kasugamycin is not expected to pose an acute risk.

2. *Chronic risk.* Using the exposure assumptions described in this unit for chronic exposure, EPA has concluded that chronic exposure to kasugamycin from food and water are below HED's LOC of 100% of the cPAD for all population subgroups. The most highly exposed population subgroup, children 1–2 years old, had a risk estimate of 1.7% cPAD. There are no residential uses for kasugamycin to aggregate with chronic exposure to kasugamycin from food and water.

3. *Short- and intermediate-term risk.* Short- and intermediate-term aggregate exposures take into account short- and intermediate-term residential exposures plus chronic exposure to food and water (considered to be a background exposure level). Because there are no residential uses for kasugamycin, kasugamycin is not expected to pose a short- or intermediate-term risk.

4. *Aggregate cancer risk for U.S. population.* Based on the lack of evidence of carcinogenicity in two adequate rodent carcinogenicity studies, kasugamycin is not expected to pose a cancer risk to humans.

5. *Determination of safety.* Based on these risk assessments, EPA concludes that there is a reasonable certainty that no harm will result to the general population or to infants and children from aggregate exposure to kasugamycin residues.

IV. Other Considerations

A. Analytical Enforcement Methodology

Adequate enforcement methodology high-performance liquid chromatography with ultraviolet detection (HPLC/UV) is available to enforce the tolerance expression. The method may be requested from: Chief, Analytical Chemistry Branch, Environmental Science Center, 701 Mapes Rd., Ft. Meade, MD 20755–5350; telephone number: (410) 305–2905; email address: *residuemethods@epa.gov.*

B. International Residue Limits

In making its tolerance decisions, EPA seeks to harmonize U.S. tolerances with international standards whenever possible, consistent with U.S. food safety standards and agricultural practices. EPA considers the international maximum residue limits (MRLs) established by the Codex Alimentarius Commission (Codex), as required by FFDCA section 408(b)(4). The Codex Alimentarius is a joint United Nations Food and Agriculture Organization/World Health Organization food standards program, and it is recognized as an international food safety standards-setting organization in trade agreements to which the United States is a party. EPA may establish a tolerance that is different from a Codex MRL; however, FFDCA section 408(b)(4) requires that EPA explain the reasons for departing from the Codex level. The Codex has not established a MRL for kasugamycin.

C. Revisions to Petitioned-For Tolerances

As EPA explained in its latest crop group rulemaking published in the **Federal Register** of August 22, 2012 (77 FR 50617) (FRL–9354–3), EPA will attempt to conform petitions seeking tolerances for crop groups to the newer established crop groups, rather than establish new tolerances under the pre-existing crop groups, as part of its effort to eventually convert tolerances for any pre-existing crop group to tolerances with coverage under the revised crop group. Therefore, although the petitioner requested tolerances for crop group 11 (pome fruit), EPA evaluated tolerances for crop group 11–10 (pome fruit).

Based on the available residue data and using the Organization for Economic Co-operation and Development (OECD) tolerance calculation procedure, EPA is establishing a tolerance of 0.20 ppm for residues of kasugamycin in or on fruit, pome (crop group 11–10).

EPA also is not establishing tolerances for walnuts and fruiting vegetables because the petitioner withdrew its tolerance requests for those commodities.

The Agency has revised the tolerance expression in 40 CFR 180.614(a) to clarify:

1. That, as provided in FFDCA section 408(a)(3), the tolerance covers metabolites and degradates of kasugamycin not specifically mentioned.

2. That compliance with the specified tolerance levels is to be determined by measuring only the specific compounds mentioned in the tolerance expression.

V. Conclusion

Therefore, tolerances are established for residues of kasugamycin, in or on pome fruits (crop group 11–10) at 0.20 ppm. This regulation additionally deletes the time-limited tolerance for apple, as the tolerance will be superseded by permanent tolerances in the various pome fruits.

VI. Statutory and Executive Order Reviews

This final rule establishes tolerances under FFDCA section 408(d) in response to a petition submitted to the Agency. The Office of Management and Budget (OMB) has exempted these types of actions from review under Executive Order 12866, entitled ''Regulatory Planning and Review'' (58 FR 51735, October 4, 1993). Because this final rule has been exempted from review under Executive Order 12866, this final rule is not subject to Executive Order 13211, entitled ''Actions Concerning Regulations That Significantly Affect Energy Supply, Distribution, or Use'' (66 FR 28355, May 22, 2001) or Executive Order 13045, entitled ''Protection of Children from Environmental Health Risks and Safety Risks'' (62 FR 19885, April 23, 1997). This final rule does not contain any information collections subject to OMB approval under the Paperwork Reduction Act (PRA) (44 U.S.C. 3501 *et seq.*), nor does it require any special considerations under Executive Order 12898, entitled ''Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations'' (59 FR 7629, February 16, 1994).

Since tolerances and exemptions that are established on the basis of a petition under FFDCA section 408(d), such as the tolerance in this final rule, do not require the issuance of a proposed rule, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*), do not apply.

This final rule directly regulates growers, food processors, food handlers, and food retailers, not States or Tribes, nor does this action alter the relationships or distribution of power and responsibilities established by Congress in the preemption provisions of FFDCA section 408(n)(4). As such, the Agency has determined that this action will not have a substantial direct effect on States or tribal governments, on the relationship between the national government and the States or tribal governments, or on the distribution of power and responsibilities among the various levels of government or between the Federal Government and Indian Tribes. Thus, the Agency has determined that Executive Order 13132, entitled ''Federalism'' (64 FR 43255, August 10, 1999) and Executive Order 13175, entitled ''Consultation and Coordination with Indian Tribal Governments'' (65 FR 67249, November 9, 2000) do not apply to this final rule. In addition, this final rule does not impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (2 U.S.C. 1501 *et seq.*).

This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA) (15 U.S.C. 272 note).

VII. Congressional Review Act

Pursuant to the Congressional Review Act (5 U.S.C. 801 *et seq.*), EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of the rule in the **Federal Register**. This action is not a ''major rule'' as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: August 20, 2014.

Marty Monell,

Acting Director, Office of Pesticide Programs.

Therefore, 40 CFR chapter I is amended as follows:

PART 180—[AMENDED]

■ 1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 321(q), 346a and 371.

■ 2. Revise § 180.614 to read as follows:

§ 180.614 Kasugamycin; tolerances for residues.

(a) *General.* Tolerances are established for residues of kasugamycin, including its metabolites and degradates, in or on the commodities listed in the following table. Compliance with the tolerance levels specified is to be determined by measuring only kasugamycin (3-*O*-[2-amino-4-[(carboxyimino-methyl)amino]-2,3,4,6-tetradeoxy-α-*D-arabino*-hexopyranosyl]-*D-chiro*-inositol) in or on the commodity.

Commodity	Parts per million
Fruit, pome, group 11–10	0.20
Vegetable, fruiting, group 8[1]	0.04

[1] There is no U.S. registration as of September 1, 2005.

(b) *Section 18 emergency exemptions.* [Reserved]

(c) *Tolerances with regional registrations.* [Reserved]

(d) *Indirect or inadvertent residues.* [Reserved]

[FR Doc. 2014–20502 Filed 8–28–14; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 271

[FRL–9915–97–Region–6; EPA–R06–RCRA–2013–0785]

Oklahoma: Final Authorization of State Hazardous Waste Management Program Revision

AGENCY: Environmental Protection Agency.

ACTION: Direct final rule.

SUMMARY: Oklahoma Department of Environmental Quality (ODEQ) has applied to the Environmental Protection Agency (EPA) for Final authorization of the changes to its hazardous waste program under the Resource Conservation and Recovery Act (RCRA). EPA has determined that these changes satisfy all requirements needed to qualify for Final authorization, and is authorizing the State's changes through this immediate final action. The EPA is publishing this rule to authorize the changes without a prior proposal because we believe this action is not controversial and do not expect comments that oppose it. Unless we receive written comments which oppose this authorization during the comment period, the decision to authorize Oklahoma's changes to its hazardous waste program will take effect. If we receive comments that oppose this action, we will publish a document in the **Federal Register** withdrawing this rule before it takes effect, and a separate document in the proposed rules section of this **Federal Register** will serve as a proposal to authorize the changes.

DATES: This final authorization will become effective on October 28, 2014 unless the EPA receives adverse written comment by September 29, 2014. If the EPA receives such comment, it will publish a timely withdrawal of this immediate final rule in the **Federal Register** and inform the public that this authorization will not take effect.

ADDRESSES: Submit your comments by one of the following methods:

1. *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the on-line instructions for submitting comments.

2. *Email: patterson.alima@epa.gov.*

3. *Mail:* Alima Patterson, Region 6, Regional Authorization Coordinator, State/Tribal Oversight Section (6PD–O), Multimedia Planning and Permitting Division, EPA Region 6, 1445 Ross Avenue, Dallas, Texas 75202–2733.

4. *Hand Delivery or Courier.* Deliver your comments to Alima Patterson, Region 6, Regional Authorization Coordinator, State/Tribal Oversight Section (6PD–O), Multimedia Planning and Permitting Division, EPA Region 6, 1445 Ross Avenue, Dallas, Texas 75202–2733.

Instructions: Do not submit information that you consider to be CBI or otherwise protected through *regulations.gov,* or email. The Federal regulations.gov Web site is an ''anonymous access'' system, which means the EPA will not know your identity or contact information unless you provide it in the body of your comment. If you send an email comment directly to the EPA without going through regulations.gov, your email address will be automatically captured and included as part of the comment that is placed in the public docket and made available on the Internet. If you submit an electronic comment, the EPA recommends that you include your name and other contact information in the body of your comment and with any disk or CD–ROM you submit. If the EPA cannot read your comment due to technical difficulties and cannot contact you for clarification, the EPA may not be able to consider your comment. Electronic files should avoid the use of special characters, any form of encryption, and be free of any defects or viruses.

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SAFETY DATA SHEET
Kasugamycin (hydrochloride)

Appendix E

Revision: 03/27/2019
Supersedes Revision: 09/30/2014

according to Regulation (EC) No. 1907/2006 as amended by (EC) No. 2015/830 and US OSHA HCS 2015

Section 1. Identification of the Substance/Mixture and of the Company/Undertaking

1.1 Product Code: 15322

Product Name: Kasugamycin (hydrochloride)

Synonyms: 3-O-[2-amino-4-[(carboxyiminomethyl)amino]-2,3,4,6-tetradeoxy-.alpha.-D-arabino-hexopyranosyl]-D-chiro-inositol, monohydrochloride;

1.2 Relevant identified uses of the substance or mixture and uses advised against:

Relevant identified uses: For research use only, not for human or veterinary use.

1.3 Details of the Supplier of the Safety Data Sheet:

Company Name: Cayman Chemical Company
1180 E. Ellsworth Rd.
Ann Arbor, MI 48108

Web site address: www.caymanchem.com

Information: Cayman Chemical Company +1 (734)971-3335

1.4 Emergency telephone number:

Emergency Contact: CHEMTREC Within USA and Canada: +1 (800)424-9300
CHEMTREC Outside USA and Canada: +1 (703)527-3887

Section 2. Hazards Identification

2.1 Classification of the Substance or Mixture:

2.2 Label Elements:

GHS Signal Word: **None**

GHS Hazard Phrases:

Based on evaluation of currently available data this substance or mixture is not classifiable according to GHS.

GHS Precaution Phrases:

No phrases apply.

GHS Response Phrases:

No phrases apply.

GHS Storage and Disposal Phrases:

Please refer to Section 7 for Storage and Section 13 for Disposal information.

2.3 Adverse Human Health Effects and Symptoms: Material may be irritating to the mucous membranes and upper respiratory tract.
May be harmful by inhalation, ingestion, or skin absorption.
May cause eye, skin, or respiratory system irritation.
To the best of our knowledge, the toxicological properties have not been thoroughly investigated.

Section 3. Composition/Information on Ingredients

CAS # / RTECS #	Hazardous Components (Chemical Name)/ REACH Registration No.	Concentration	EC No./ EC Index No.	GHS Classification
19408-46-9 NM7521800	Kasugamycin (hydrochloride)	100.0 %	606-307-1 NA	No data available.

Section 4. First Aid Measures

4.1 Description of First Aid Measures:

In Case of Inhalation: Remove to fresh air. If not breathing, give artificial respiration or give oxygen by trained personnel. Get immediate medical attention.

In Case of Skin Contact: Immediately wash skin with soap and plenty of water for at least 15 minutes. Remove contaminated clothing. Get medical attention if symptoms occur. Wash clothing before reuse.

In Case of Eye Contact: Hold eyelids apart and flush eyes with plenty of water for at least 15 minutes. Have eyes examined and tested by medical personnel.

In Case of Ingestion: Wash out mouth with water provided person is conscious. Never give anything by mouth to an unconscious person. Get medical attention. Do NOT induce vomiting unless directed to do so by medical personnel.

Section 5. Fire Fighting Measures

5.1 Suitable Extinguishing Media: Use alcohol-resistant foam, carbon dioxide, water, or dry chemical spray.
Use water spray to cool fire-exposed containers.

Unsuitable Extinguishing Media: A solid water stream may be inefficient.

5.2 Flammable Properties and Hazards: No data available.

No data available.

Flash Pt: No data.

Explosive Limits: LEL: No data. UEL: No data.

Autoignition Pt: No data.

5.3 Fire Fighting Instructions: As in any fire, wear self-contained breathing apparatus pressure-demand (NIOSH approved or equivalent), and full protective gear to prevent contact with skin and eyes.

Section 6. Accidental Release Measures

6.1 Protective Precautions, Protective Equipment and Emergency Procedures: Avoid raising and breathing dust, and provide adequate ventilation.
As conditions warrant, wear a NIOSH approved self-contained breathing apparatus, or respirator, and appropriate personal protection (rubber boots, safety goggles, and heavy rubber gloves).

6.2 Environmental Precautions: Take steps to avoid release into the environment, if safe to do so.

6.3 Methods and Material For Containment and Cleaning Up: Contain spill and collect, as appropriate.
Transfer to a chemical waste container for disposal in accordance with local regulations.

Section 7. Handling and Storage

7.1 Precautions To Be Taken in Handling: Avoid breathing dust/fume/gas/mist/vapours/spray.
Avoid prolonged or repeated exposure.

7.2 Precautions To Be Taken in Storing: Keep container tightly closed.
Store in accordance with information listed on the product insert.

Section 8. Exposure Controls/Personal Protection

8.1 Exposure Parameters:

8.2 Exposure Controls:

8.2.1 Engineering Controls (Ventilation etc.): Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below recommended exposure limits.

8.2.2 Personal protection equipment:

Eye Protection: Safety glasses

Protective Gloves: Compatible chemical-resistant gloves

Other Protective Clothing: Lab coat

Respiratory Equipment (Specify Type): NIOSH approved respirator, as conditions warrant.

Work/Hygienic/Maintenance Practices: Do not take internally.
Facilities storing or utilizing this material should be equipped with an eyewash and a safety shower.
Wash thoroughly after handling.

No data available.

Section 9. Physical and Chemical Properties

9.1 Information on Basic Physical and Chemical Properties

Physical States:	[ ] Gas [ ] Liquid [X] Solid
Appearance and Odor:	A crystalline solid
pH:	No data.
Melting Point:	No data.
Boiling Point:	No data.
Flash Pt:	No data.
Evaporation Rate:	No data.
Flammability (solid, gas):	No data available.
Explosive Limits:	LEL: No data. UEL: No data.
Vapor Pressure (vs. Air or mm Hg):	No data.
Vapor Density (vs. Air = 1):	No data.
Specific Gravity (Water = 1):	No data.
Solubility in Water:	No data.
Solubility Notes:	~5 mg/ml in PBS (pH 7.2);
Octanol/Water Partition Coefficient:	No data.
Autoignition Pt:	No data.
Decomposition Temperature:	No data.
Viscosity:	No data.

9.2 Other Information

Percent Volatile:	No data.
Molecular Formula & Weight:	C14H25N3O9 • HCl 415.8

Section 10. Stability and Reactivity

10.1 Reactivity: No data available.

10.2 Stability: Unstable [ ] Stable [X]

10.3 Stability Note(s): Stable if stored in accordance with information listed on the product insert.

Polymerization: Will occur [ ] Will not occur [X]

10.4 Conditions To Avoid: No data available.

10.5 Incompatibility - Materials To Avoid: strong oxidizing agents

10.6 Hazardous Decomposition or Byproducts: carbon dioxide
carbon monoxide
hydrogen chloride gas
nitrogen oxides

Section 11. Toxicological Information

11.1 Information on Toxicological Effects: The toxicological effects of this product have not been thoroughly studied.
Kasugamycin (hydrochloride) - Toxicity Data: Oral LD50 (rat): 22 g/kg; Intraperitoneal LD50 (rat): 12 g/kg; Subcutaneous LD50 (rat): 17 g/kg; Oral LD50 (mouse): 20500 mg/kg; Intraperitoneal LD50 (mouse): 7600 mg/kg; Subcutaneous LD50 (mouse): 12 g/kg;

Chronic Toxicological Effects: Kasugamycin (hydrochloride) - Investigated as an agricultural chemical.
Only select Registry of Toxic Effects of Chemical Substances (RTECS) data is presented here. See actual entry in RTECS for complete information.
Kasugamycin (hydrochloride) RTECS Number: NM7521800

CAS #	Hazardous Components (Chemical Name)	NTP	IARC	ACGIH	OSHA
19408-46-9	Kasugamycin (hydrochloride)	n.a.	n.a.	n.a.	n.a.

Section 12. Ecological Information

12.1 Toxicity: Avoid release into the environment.
Runoff from fire control or dilution water may cause pollution.

12.2 Persistence and Degradability: No data available.

12.3 Bioaccumulative Potential: No data available.

12.4 Mobility in Soil: No data available.

12.5 Results of PBT and vPvB assessment: No data available.

12.6 Other adverse effects: No data available.

Section 13. Disposal Considerations

13.1 Waste Disposal Method: Dispose in accordance with local, state, and federal regulations.

Section 14. Transport Information

14.1 LAND TRANSPORT (US DOT):

DOT Proper Shipping Name: Not dangerous goods.

DOT Hazard Class:

UN/NA Number:

14.1 LAND TRANSPORT (European ADR/RID):

ADR/RID Shipping Name: Not dangerous goods.

UN Number:

Hazard Class:

14.3 AIR TRANSPORT (ICAO/IATA):

ICAO/IATA Shipping Name: Not dangerous goods.

Additional Transport Information: Transport in accordance with local, state, and federal regulations.

Section 15. Regulatory Information

EPA SARA (Superfund Amendments and Reauthorization Act of 1986) Lists

CAS #	Hazardous Components (Chemical Name)	S. 302 (EHS)	S. 304 RQ	S. 313 (TRI)
19408-46-9	Kasugamycin (hydrochloride)	No	No	No

CAS #	Hazardous Components (Chemical Name)	Other US EPA or State Lists
19408-46-9	Kasugamycin (hydrochloride)	CAA HAP,ODC: No; CWA NPDES: No; TSCA: No; CA PROP.65: No

Regulatory Information Statement: This SDS was prepared in accordance with 29 CFR 1910.1200 and Regulation (EC) No.1272/2008.

Section 16. Other Information

Revision Date: 03/27/2019

Additional Information About This Product: No data available.

Company Policy or Disclaimer: DISCLAIMER: This information is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes.

Appendix F: Kasugamycin References

* = full text attached as an Appendix

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Annual Report – 2018-19 Appendix G

Prepared for the California Apple commission

Project Title:	Evaluation of new biological controls for management of fire blight of apples caused by *Erwinia amylovora* and evaluation of new natural products as organic postharvest fungicides for pome fruits
Project Leader:	Dr. J. E. Adaskaveg, Department of Plant Pathology and Microbiology, University of California, Riverside CA 92521.
Cooperators:	D. Thompson, D. Cary, and H. Förster

SUMMARY

Fire blight management

1. Antibiotic and copper resistance surveys for populations of *Erwinia amylovora* in California pear growing areas were continued in 2018.
   a. Kasugamycin: All 70 strains from 13 orchard locations in Sacramento and Lake Co. were sensitive.
   b. Streptomycin: Resistance was detected in all but one location. Forty-two of the resistant strains had plasmid-based moderate resistance (MIC <20 ppm) and 19 strains displayed high resistance that most likely was chromosomal-based. Thus, populations of *E. amylovora* re-adjust rapidly to selection pressure (i.e., bactericide applications). Streptomycin should be used strategically, and these findings stress the importance of resistance management with mixtures or rotations and that new alternatives need to be developed.
   c. Oxytetracycline: For the first time, high levels of resistance with growth at >40 ppm were detected at two locations. These resistant strains were also highly resistant to streptomycin. In the location with the highest incidence of Oxy^R, nine applications of the antibiotic were applied between 2017 and 2018. Oxytetracycline resistance in *E. amylovora* has never been reported previously at this level, and this finding is a serious concern. Currently, it is not known if these resistant strains are competitively fit and will persist in the absence of selection pressure.
   d. Copper: Moderate copper resistance was present in strains of *E. amylovora*. Growth was similar to the control using 20 ppm MCE and was reduced at 30 ppm MCE on nutrient agar. Spontaneous mutants growing at high concentrations of copper were also observed. Management failures with the use of copper under high disease pressure have been attributed to highly favorable environments, low rates of copper registered, moderate copper resistance, and spontaneous mutants with high copper resistance.
   e. In 30-min direct exposures of *E. amylovora* suspensions to the food preservatives nisin or ε-poly-L-lysine, the toxicity of both compounds was significantly increased with the addition of ethylenediaminetetraacetic acid (EDTA).
2. Field trials on the management of fire blight were conducted under high disease pressure on cvs. Granny Smith and Fuji apple, as well as under low disease pressure on Bartlett pear.
   a. On Granny Smith apple, Blossom Protect with the newly formulated buffer additive was the most effective treatment. The rotation treatment of Badge, Badge + ProPhyt, followed by two applications of Blossom Protect, however, was somewhat less effective. Statistically similarly effective treatments to Blossom Protect were Kasumin + FireWall, Kasumin + ε-poly-L-lysine + zinc oxide, as well as Kasumin 2L and Kasumin 4L by themselves. Nisin and ε-poly-L-lysine by themselves were only moderately effective, and the addition of EDTA and zinc oxide to nisin or of Dart (28.3% capric and 41.7% caprylic acids) to ε-poly-L-lysine resulted in numerical but not statistical increases in efficacy.
   b. On Fuji apple, Kasumin 2L and 4L, and FireLine + zinc oxide + Dart provided the highest efficacy among treatments evaluated. Among treatments containing the preservatives nisin or ε-poly-L-lysine, ε-poly-L-lysine + zinc oxide + EDTA was most effective.
   c. In small-scale studies, two new experimental biocontrol agents were not effective in reducing fire blight.
   d. Kasumin is currently considered a conventional treatment, however, efforts are underway to obtain an organic registration. The compound is a natural substance that is commercially produced by fermentation. In contrast to streptomycin and oxytetracycline, it has very minimal or no usage in human medicine.

Postharvest decay control

1. In laboratory studies on control of blue mold and gray mold of apple, the high-solubility formulation of natamycin was numerically more effective than the BioSpectra formulation. Natamycin again was not effective in reducing blue mold of pears, and there were differences in efficacy also among sources of apple fruit that may be related to the fruit age (time of storage after harvest).
2. In an experimental packingline study using in-line drench applications, BioSpectra significantly reduced blue mold and gray mold of Granny Smith apple, but a treatment with 300 ppm Scholar was significantly more effective.
3. The efficacy of natamycin needs to be improved for apples and other pome fruits. Although efficacy of Scholar or Academy is not improved when BioSpectra is added in mixture treatments, natamycin represents a resistance management strategy. Resistance to natamycin has not been reported previously to any *Penicillium* species, although the compound has been registered for food uses for over 20 years.

INTRODUCTION

Epidemiology and management of fire blight. Fire blight, caused by the bacterium *Erwinia amylovora,* is one of the most destructive diseases of pome fruit trees including apples. Current control programs are based on protective schedules because available compounds are contact treatments and are not systemic except for the antibiotic streptomycin. Registered treatments include copper products, antibiotics, as well as natural products and biocontrol agents. Conventional copper compounds are only effective when disease severity is low to moderate. They may cause fruit russeting and therefore, labeled rates are at low amounts of metallic copper equivalent (MCE) that are at the limit of effectiveness. New re-formulated copper products that can be used at reduced MCE rates and that cause less phytotoxicity are available. Some products are OMRI-approved including Badge X2, CS-2005, and Cueva. Because only few treatments are permitted for organic apple production, research on OMRI-approved coppers needs to be continued, and some were included in our 2018 field studies. In our surveys, however, we detected low to moderate levels of copper insensitivity in pathogen populations.

The antibiotics streptomycin and oxytetracycline can only be used in conventional pome fruit production. The incidence of resistance to streptomycin in California orchards has been fluctuating from very high to low in our surveys between 2006 and 2017. Reduced sensitivity to oxytetracycline has only been found sporadically, and these isolates did not persist. Kasugamycin (Kasumin) is now registered in California. Resistance to kasugamycin in *E. amylovora* has not been found to date. Efforts are ongoing to differentiate kasugamycin from other bactericides and allow certification as an organic treatment by the National Organic Standards Board and OMRI.

The biocontrol treatments Blight Ban A506 (*Pseudomonas fluorescens* strain A506) and Bloomtime Biological (*Pantoea agglomerans* strain E325), and the fermentation product of *Bacillus subtilis* Serenade (strain QST 713) have been inconsistent over the years in their performance in our trials and were most effective under low inoculum levels and less favorable micro-environments. The biocontrol Blossom Protect (*Aureobasidium pullulans*) has been very effective under less to moderately favorable disease conditions, and it is one of the most consistent biologicals that we have evaluated. Biocontrols are most effective when they are actively growing on the plant. A new buffer additive for Blossom Protect that was developed to increase growth of the biocontrol agent became available in 2019 and was included in our field studies. We are also evaluating other bactericide alternatives such as the natural fermentation compounds lactic acid, ε-poly-L-lysine, and nisin that have known anti-bacterial activity and are used as food preservatives. They potentially could qualify for organic production. Our initial evaluations with these compounds showed high toxicity in lab studies, but only moderate activity in the field. Therefore, we continue to try to improve their efficacy by using selected additives. Our goal is to develop effective rotational programs for organic farming practices with the use of copper and biologicals, as well as conventional programs with the use of antibiotics, copper, biologicals, and other bactericidal compounds for use during bloom and early fruit development.

Management of postharvest decays. Apples like other pome fruits can be stored for some period of time in optimum fruit storage environments. Still, postharvest decays caused by fungal organisms can result in economic crop losses. The major postharvest pathogens of apples are *Penicillium expansum, Botrytis cinerea, Alternaria*

alternata, Mucor piriformis, and *Neofabraea* spp. causing blue mold, gray mold, Alternaria rot (black mold), Mucor decay, and bull's eye rot, respectively. There is a deficiency in postharvest biocontrols and natural products that are available for preventing these decays in storage. BioSave 100 is one of the few materials currently available in the United States, but its efficacy is limited. Still, other biological products are registered in other countries and these potentially could be evaluated for California conditions if registrants decide to market their products (e.g., Shemer - *Metschnikowia fructicola*, Candifruit - *Candida sake*, Nexy - *Candida oleophila*, Boni-Protect - *Aureobasidium pullulans*) in the U.S.

We previously showed that the bio-fungicide polyoxin-D (Ph-D, Oso, Tavano) is very effective in reducing gray mold and Alternaria rot, but not blue mold. Polyoxin-D was approved as an organic fungicide by the NOSB in April 2018 and is currently pending pre-harvest labeling and postharvest registration on multiple crops. We also demonstrated the efficacy of another bio-fungicide, natamycin (pimaricin). For many years, natamycin has been a federally-approved food additive to prevent mold growth, including *Penicillium* species, on dairy and meat products in the United States and other countries. Over this time, resistance in *Penicillium* species against natamycin has not occurred. This compound was registered in late 2016 as BioSpectra for postharvest treatment of citrus and stone fruits. Natamycin has an exempt registration status and has been submitted to the NOSB for organic registration. In our evaluations, natamycin showed very good and consistent efficacy against gray mold and Mucor rot. Efficacy against blue mold, however, has been very variable over the years ranging from excellent to unsatisfactory. Therefore, our goal is to improve its performance so it potentially can be made available to the pome fruit industry. In 2018/19, we continued to compare several formulations of natamycin, and we tried to determine the causes for its inconsistency.

OBJECTIVES FOR 2018-2019

Fire blight research

1. Evaluate the efficacy of treatments for managing fire blight.
   A. Evaluate growth enhancers (e.g., buffers) of biological control agents in lab and field trials.
   B. Laboratory in vitro tests on copper and zinc products (registered copper products) with newly identified antibacterial, food additives (lactic acid, poly-L-lysine, and nisin) and experimental compounds (SBH derivatives) that enhance the activity of copper and possibly zinc.
   D. Field trials with protective air-blast spray treatments:
      i. Kasugamycin in combination with organic treatments to support organic petition to NOSB.
      ii. New formulations of copper (e.g., Badge X2, CS-2005, Cueva) and SBH as a copper activity enhancer in combination or rotation with newly identified antibacterial, food additives (lactic acid, poly-L-lysine, and nisin).
      ii. Biological treatments (Blossom Protect, Serenade) with and without the addition of growth enhancers.
      iii. Blockers of bacterial infection that interfere with Type III secretion systems (e.g., TS products) alone or in mixtures with other biological control treatments.

Postharvest research

2. Comparative evaluation of new postharvest fungicides
   A. Evaluate natamycin (BioSpectra) and other new postharvest fungicides such as Academy at selected rates against gray mold, blue mold, Alternaria decay, and bull's eye rot and compare to fludioxonil.
   B. Evaluate mixtures of these compounds.

PLANS AND PROCEDURES

Isolation and culturing of* E. amylovora *and sensitivity testing against antibiotics and copper. Fire blight samples were obtained from pome fruit trees in the spring of 2018 from commercial orchards. Infected plant material was surface-disinfested for 1 min using 400 mg/L sodium hypochlorite, rinsed with sterile water, cut into small sections, and incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to stream out of the tissue. Suspensions were streaked onto yeast extract-dextrose-$CaCO_3$ agar (YDC). Single colonies were transferred and the identity of the isolates as *E. amylovora* was verified by colony morphology and by PCR using primers specific for *E. amylovora* (Appl. Environ. Microbiol. 58:3522-2536). Strains were tested for their sensitivity to streptomycin and oxytetracycline using the spiral gradient dilution (SGD) method. Copper

sensitivity of strains was determined by streaking bacterial suspensions (70% transmission at 600 nm) on CYE (casitone, yeast extract, glycerol) or nutrient agar amended with 0, 20, 30, or 40 ppm MCE. Growth was recorded after 2 days of incubation at 25C and was rated as +++ (growth not inhibited, similar to the control), ++ (growth inhibited as compared to the control), or + (growth sparse).

The toxicity of ε-poly-L-lysine and nisin against *E. amylovora* was evaluated in direct contact assays. For this, suspensions of a strain of *E. amylovora* were incubated in final concentrations of 500 ppm of these antimicrobials, and water was used in control treatments. To possibly improve the toxicity of ε-poly-L-lysine and nisin, ethylenediaminetetraacetic acid (EDTA) was added using selected concentrations. Mixtures were incubated for 30 min, diluted 1:1000 with sterile water, and aliquots were then plated onto nutrient agar. After 2 days, bacterial colonies were enumerated, and percent inhibition in colony formation as compared to the control was calculated.

Field studies on the management of fire blight using protective treatments. Air-blast field studies on the relative efficacy of protective treatments were conducted in experimental cvs. Granny Smith and Fuji apple orchards at the Kearney Agricultural Research and Extension Center (KARE). All trees received a copper treatment at bud break to help reduce the high amount of inoculum present in these orchards that made evaluation of bactericide treatments difficult in the last couple of years. Four applications were done starting at 5-10% bloom and followed by phenology-based treatments until petal fall. Treatments included single treatments, mixtures, and a rotation. Incidence of blight was assessed in late May based on the number of infected flower clusters of approximately 200 clusters evaluated for each of the four two-tree replications. Additionally, potential phytotoxic effects of the treatments (e.g., fruit russeting and leaf burn) were evaluated. For comparison, field studies were also conducted on Bartlett pear with some overlapping treatments to the apple studies. Four applications were done, and disease was evaluated in early May. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

In small-scale field studies at UC Davis, two new experimental biocontrol agents (coded BC250 and T3-07) were evaluated. Treatments were applied to open flowers of Fuji apple or Comice pear using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3.5 h, and peroxyacetic acid (e.g., Oxidate) was applied as a secondary treatment to some of the primary treatments after another hour. Streptomycin was used as standard treatment for comparison. Disease was evaluated after 1 week. Data were statistically analyzed as described above.

Efficacy of new postharvest fungicides for managing apple decays in storage. A new high-solution formulation of natamycin was compared to the BioSpectra formulation using Granny Smith apple inoculated with *P. expansum* or *B. cinerea*. The new formulation was also evaluated for its efficacy to control blue mold of Granny Smith apple, Shinko apple pear, as well as Bartlett, D'Anjou, and Bosc pear in the laboratory. Fruit were treated using an air-nozzle sprayer after 12 h and then incubated for 7 days at 20C.

Granny Smith fruit that were treated similar to commercial practices concerning harvest, handling, packing, and temperature-management of fruit were used in an experimental packingline study at KARE. Fruit were wound-inoculated with conidial suspensions of *B. cinerea* or *P. expansum* and treated after 15 to 16 h with BioSpectra or Scholar by in-line drenches that were followed by a CDA application with a carnauba-based fruit coating (i.e., Decco 230). For each of four replications, 24 fruit were used. Data were analyzed using analysis of variance, and averages were separated using least significant difference mean separation procedures of SAS 9.4.

RESULTS AND DISCUSSION

Survey of antibiotic and copper sensitivity in* E. amylovora *strains from California. In 2018, 70 strains were obtained from 13 orchard locations in Sacramento Co. and tested. All strains were found to be sensitive to **kasugamycin** (Table 1). Resistance to **streptomycin** was detected in all but one location. A low incidence of resistance (2 of 6 isolates) was present in an orchard where only copper and Serenade were applied for fire blight management. Forty-two of the resistant strains from the survey had plasmid-based moderate resistance (MIC <20 ppm) and 19 strains displayed high resistance that most likely was chromosomal-based. For these latter strains streptomycin concentrations of up to 40 ppm were tested, but based on our previous results, these strains typically still grow at >2000 ppm. In one location all 6 resistant strains, and in another location, 6 of the 7 strains were highly resistant. This high incidence of high resistance is interesting because in our surveys several years ago, high-resistance was present only at very low levels. Thus, as we demonstrated

previously, the occurrence of streptomycin resistance fluctuates widely among years and probably reflects strain fitness and antibiotic use. Overall, there was no clear correlation between streptomycin usage in 2018 and the incidence and level of streptomycin resistance that was present in the pathogen population (Table 1). However, the previous seasons' applications possibly also need to be considered that will determine the composition of the overwintering pathogen population.

Results over the years support our recommendation that streptomycin can be used once a year effectively for most growers. In years with high- to moderate disease levels, pathogen populations exposed to multiple applications of streptomycin will be under selection pressure of the antibiotic, and this will allow re-emergence of resistant sub-populations.

In our evaluations of **oxytetracycline** toxicity against *E. amylovora* strains from the 13 orchard locations, surprisingly we detected high levels of resistance with growth at >40 ppm in the spiral gradient endpoint assay at two locations (6 of 7 strains tested in one orchard and 1 of 8 strains tested in another orchard; Table 1). These resistant strains were also highly resistant to streptomycin. In the location with the highest incidence of Oxy^R, nine applications of the antibiotic were applied between 2017 and 2018. High dependency on one antibiotic in a two-year period may be responsible for the selection of the resistance detected. The strains' identity was verified as *E. amylovora* by specific PCR primers, and their resistance was confirmed by culturing on nutrient agar amended with 40 ppm oxytetracycline (Table 1). Oxytetracycline resistance in *E. amylovora* has never been reported previously at this high level, and this finding is of serious concern. Considering the wide fluctuations in streptomycin resistance in California pear orchards and the previously described non-persistent population of the pathogen with reduced sensitivity to oxytetracycline, it is currently not known if these new resistant strains are competitively fit and will persist in the absence of selection pressure (i.e., applications with oxytetracycline and streptomycin). We plan to characterize these strains genetically to determine if oxytetracycline resistance genes are similar to those that were previously described from other bacteria (non-plant pathogens).

Regarding **copper** sensitivity, growth of all 70 strains was completely inhibited on CYE (a growth medium with a low copper-binding capacity) agar amended with 20 ppm MCE (Table 1). All strains grew on the nutrient-rich nutrient agar at 20 ppm MCE similar as on non-amended agar. At 30 ppm MCE on nutrient agar, confluent growth of most strains was reduced or inhibited. Thus, as in 2015- 2017, current *E. amylovora* populations are considered moderately copper-resistant. Again, we observed the frequent presence of spontaneous mutant colonies emerging at higher copper concentrations. These mutants were not stable when sub-cultured on copper-free media and reverted back to sensitivity. If these mutants also occur in the field, however, under continued presence of selection pressure (i.e., copper sprays) they may successfully compete and cause disease.

Previously, we outlined several factors that likely contributed to the failure of copper applications to control fire blight. Here, we re-summarize this information: 1) Highly conducive disease conditions may allow the pathogen to overcome the suppressive action of copper (copper is bacteriostatic and does not kill the pathogen); 2) Only low rates of copper are registered for fire blight management; 3) There is moderate copper resistance in *E. amylovora;* and 4), Selection of populations (spontaneous mutants) with higher copper resistance after repeated applications may lead to disease in the presence of copper. Fruit russetting also may occur on some pome fruit varieties with repeated applications of copper. Therefore, there is a need to develop and register products that have different modes of action and that potentially can be registered as organic products.

***In vitro toxicity of ε-poly-L-lysine and nisin against* E. amylovora.** In 30-min direct exposures of *E. amylovora* suspensions, colony formation was reduced by 40 or 50% using nisin or ε-poly-L-lysine, respectively (Fig. 1). The toxicity of both food additives was significantly increased with the addition of 100 or 500 ppm EDTA. Growth was completely inhibited by adding 500 ppm EDTA to either bactericide and by approximately 80 or 100% using 100 ppm EDTA with ε-poly-L-lysine or nisin, respectively; EDTA by itself was only moderately or not inhibitory, depending on the rate used. These results indicated that the toxicity of nisin and ε-poly-L-lysine could be increased, and this was subsequently evaluated in field efficacy studies.

***Field studies on fire blight using protective treatments*.** Fire blight incidence in our research plots in the spring of 2018 was high on apple, i. e., over 40% based on infected flower clusters of untreated control trees. Disease,

however, was low on Bartlett pear due to cool temperatures during bloom time. The latter orchard location often had very high disease levels over the years. On Granny Smith apple, Blossom Protect with the newly formulated buffer additive was the most effective treatment, and disease incidence was reduced from 42.4% in the control to 11.1% (Fig. 2). The rotation treatment of Badge, Badge + ProPhyt, followed by two applications of Blossom Protect, however, was somewhat less effective. Statistically similarly effective treatments to Blossom Protect were Kasumin + FireWall, Kasumin + ε-poly-L-lysine + zinc oxide, as well as Kasumin 2L and Kasumin 4L by themselves. Nisin and ε-poly-L-lysine by themselves were only moderately effective, and the addition of EDTA and zinc oxide to nisin or of Dart (28.3% capric and 41.7% caprylic acids) to ε-poly-L-lysine only resulted numerical but not statistically significant increases in efficacy. The three organic copper products Cueva, Mastercop, and CS-2005 also had moderate activity, with Cueva being the most effective. No phytotoxicity was observed using any of the treatments.

On Fuji apple, Kasumin 2L and 4L, and FireLine + zinc oxide + Dart provided the highest efficacy among treatments evaluated (Fig. 3). Among treatments containing the preservatives nisin or ε-poly-L-lysine, ε-poly-L-lysine + zinc oxide + EDTA was most effective. In the latter mixture treatment, ε-poly-L-lysine at the lower rate of 3.5 oz was more effective that at the 13.5-oz rate. Interestingly, in vitro direct exposure studies also indicated that this compound was more toxic at lower rates used. Thus, this needs to be further explored. ε-poly-L-lysine is a very large molecule, and lower concentrations possibly have better access to target sites and prevent auto-binding to itself. Two new potential biocontrol agents that showed activity in vitro were provided to us and were evaluated in small-scale studies on Fuji apple and Comice pear trees. In contrast to treatments with streptomycin, the incidence of blighted flowers as compared with the control was not reduced using these bacteria (Fig. 4). We followed a protocol specified by the provider of the bacteria, and a different treatment-inoculation schedule (e.g., longer time between treatment and inoculation) may improve the effectiveness. These results also stress the difficulty in making potential biocontrol agents that show activity in the lab to be effective treatments in the field.

In a field trial on Bartlett pear, all treatments evaluated significantly reduced the disease from the control, mostly to low levels (Fig. 5). Kasumin + FireWall showed the least amount of blight. With a disease incidence in the control of less than 8%, Serenade + Cueva and Nisin + EDTA + zinc oxide also performed well, however, Nisin + EDTA was the least effective treatment. Three new experimental treatments (NSA, NS1, and NS2) had moderate to good efficacy, and the best one (i.e., NS2) should be evaluated at higher disease pressure).

In our spring 2019 field trials on the management of fire blight, numerous compounds were evaluated that often were used in mixtures. Because it was not possible to test each mixture compound by itself in a field study with trees, it is often difficult to determine which of the mixture components improved efficacy, especially when triple mixtures were used. Some conclusions, however, can be made. ε-poly-L-lysine and nisin have potential as fire blight management treatments, especially considering that they are currently have generally regarded as safe (GRAS) status as food additives by the US Food and Drug Administration (FDA). Zinc oxide did not improve the efficacy of Mycoshield + LI700 at the rate evaluated but improved the efficacy of the Nisin + EDTA treatment (Fig. 5). Formulations are very difficult to develop and require expertise from formulation chemists. As indicated above, lower rates of these components may be better in combination with the active ingredient than higher rates. Thus, we are pursuing development of formulations in cooperation with a potential registrant. Developing these new modes of action is critical in providing safe, effective alternatives to current products registered and for reducing the risk of resistance development to existing registered products as rotational or mixture treatments.

In conclusion, among organic treatments, only Blossom Protect showed acceptable commercial efficacy in the management of fire blight similar to standards. Conventional treatments containing the antibiotics streptomycin or kasugamycin were always very effective. Still, other biological treatments to be considered are the liquid copper formulation Cueva and the preservatives nisin and ε-poly-L-lysine. Formulating these antimicrobial food preservatives to improve their efficacy needs to be done in cooperation with a potential registrant. Nisin and ε-poly-L-lysine are eligible for biopesticide registration with the US-EPA. Kasumin is currently considered a conventional treatment, however, efforts are underway to obtain an organic registration. The compound is a natural substance that is commercially produced by fermentation of *Streptomyces* species. In contrast to streptomycin and oxytetracycline, it has very minimal or no usage in human and veterinary medicine. Thus, an organic registration seems plausible.

Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures. Postharvest studies focused on the efficacy of the new natural compound natamycin that is currently registered as a biopesticide with tolerance exemption status by the US-EPA. The fungicide is registered as BioSpectra on citrus and stone fruits. In laboratory studies, we compared the efficacy of two formulations for the control of blue mold and gray mold, the commercial BioSpectra and a high-solubility solution formulation. Previously, we determined that the WP formulation is generally less effective. In this year's studies, the high-solubility formulation was numerically more effective as shown in Fig. 6 for a study on Granny Smith apple (and also in other trials on pome and other fruits that are not presented here). For blue mold control, we found natamycin to be highly effective on Granny Smith apple, but not on apple pear and three pear cultivars (Fig. 7). We noted this difference in efficacy among pome fruit cultivars previously, and control of blue mold of pears with natamycin has been a challenge for several years because the fungicide is highly effective on other decays such as gray mold, Alternaria, and Rhizopus rot (even when inoculated on the same fruit that are also inoculated with *P. expansum*). In doing numerous studies over early to late fall, we also noted differences in efficacy of natamycin among sources of apple fruit that likely were related to the fruit age (time of storage after harvest). Thus, late-season tests with Granny Smith apple were generally not very successful, although Scholar still was effective. We are planning to do all our postharvest studies with natamycin soon after harvest in 2019 when commercial postharvest treatments are mostly done in California.

In an experimental packingline study using in-line drench applications, BioSpectra significantly reduced blue mold and gray mold of Granny Smith apple, and the 1000-ppm rate was more effective than the 500-ppm rate for blue mold (Fig. 8). A treatment with 300 ppm Scholar was significantly more effective than those with BioSpectra. Based on the moderate efficacy of natamycin, natamycin may not become registered on pome fruits unless it is developed in a premixture with other fungicides. Still, we will continue to try to improve its efficacy. Moreover, natamycin still has a chance to receive an OMRI listing with our NOSB petition. Mixtures of BioSpectra with Scholar or Academy were evaluated previously by us and were very effective against blue mold, gray mold, Alternaria rot and bull's eye rot. Although efficacy is not improved as compared to using the two registered fungicides by themselves, adding natamycin represents an excellent resistance management strategy. Resistance to natamycin has not been reported previously to any *Penicillium* species, although the compound has been registered for food uses for over 20 years.

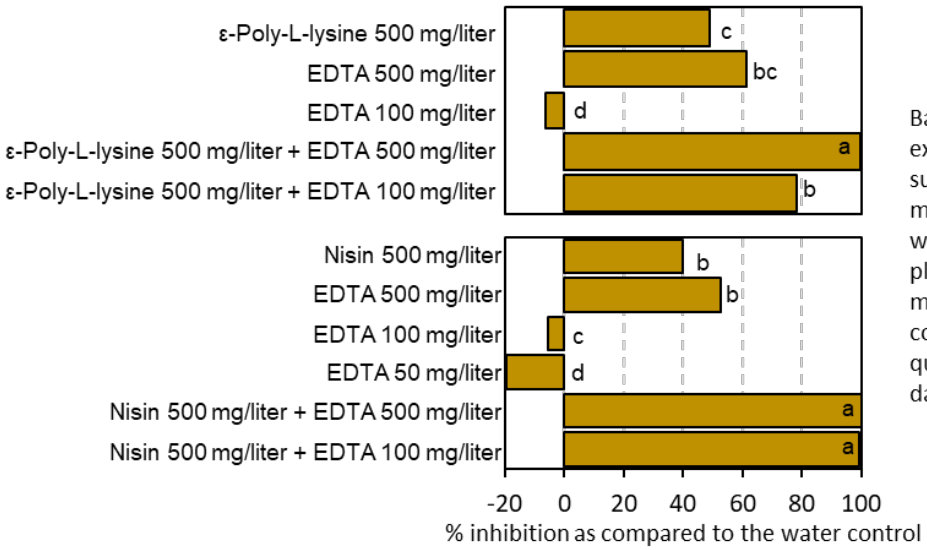
Table 1. Sensitivity of *E. amylovora* strains from pear orchards in Sacramento Co. to streptomycin, oxytetracycline, kasugamycin, and copper in 2018

				Copper				
	Strepto-	Oxytetra-	Kasuga-	CYE	Nutrient agar			
Location	mycin	cycline	mycin	20 ppm	20 ppm	30 ppm	40 ppm	Chemical spray program
1	MR	S	S	--	++	+*	-	Oxy (3)- Strep (1) rotation
	HR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
2	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	Oxy (3)- Strep (1) rotation
	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	HR	HR	S	--	+++	*	-	
3	HR	S	S	--	+++	*	-	Oxy (4)- Strep (1)- PO3 (2) rotation
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	+*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
4	MR	S	S	--	+++	+*	-	Oxy (4)- Strep (1)- PO3 (2) rotation
	MR	S	S	--	+++	+*	-	
5	MR	S	S	--	+++	+*	-	Oxy (4)- Strep (1)- PO3 (2) rotation
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
6	S	S	S	--	+++	++	*	Oxy (4)- Strep (1)- PO3 (2) rotation
	S	S	S	--	+++	++	*	
	S	S	S	--	+++	++	*	
	S	S	S	--	+++	++	*	
7	MR	S	S	--	+++	*	-	Copper, Oxy-Strep mixtures, rotated with Strep, Kasu
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	++*	*	
8	MR	S	S	--	+++	*	-	Copper, Oxy-Strep mixtures, rotated with Strep, Kasu
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
9	MR	S	S	--	+++	*	-	Copper, Oxy-Strep mixtures, rotated with Strep, Kasumin
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	+*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
10	HR	S	S	--	+++	*	-	Oxy (3)- Strep (2) rotation
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
11	MR	S	S	--	+++	*	-	Oxy (3)- Strep (2) rotation
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	*	
	MR	S	S	--	+++	*	*	
	MR	S	S	--	+++	++	*	
12	MR	S	S	--	+++	++	*	Oxy-Strep mixtures
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	S	S	S	--	+++	++	*	
	HR	HR	S	--	+++	++*	*	
	MR	S	S	--	+++	+++	*	
13	S	S	S	--	+++	++*	*	Copper, Serenade
	S	S	S	--	+++	*	*	
	S	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	S	S	S	--	+++	+*	*	
	MR	S	S	--	+++	*	*	

Sensitivity to streptomycin, oxytetracycline, and kasugamycin was determined using the spiral gradient endpoint method. S = sensitive, MR = moderately resistant (MIC = <20 ppm), HR = highly resistant (MIC = >40 ppm).

Sensitivity to copper was determined by growth on amended CYE (casitone, yeast extract, glycerol agar) or nutrient agar. Copper ratings: +++ = growth similar to non-amended agar, ++ = reduced growth, + = little growth, - = no growth. * = Spontaneous mutants growing, but no confluent growth.

Fig. 1. In vitro toxicity of ε-poly-L-lysin and nisin against *E. amylovora* - Direct exposure studies



Bacteria were exposed to the test substances for 30 min, diluted 1:100 with water, and then plated onto agar media. Bacterial colonies were quantified after 2 days.

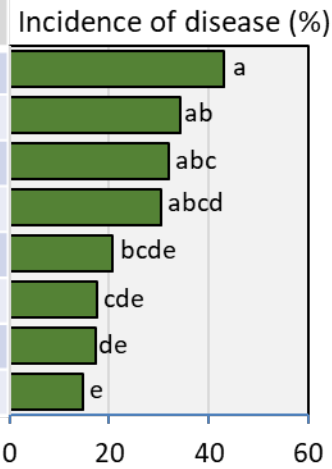
Fig. 2. Efficacy of new mostly organic bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2019

	Treatment	Rate	3-30	4-4	4-10	4-19
	Control	---	---	---	---	---
	ε-Poly-L-lysine	3.5 oz	@	@	@	@
	MasterCop	64 fl oz	@	@	@	@
	CS-2005	25 fl oz	@	@	@	@
	Nisin	13.5 oz	@	@	@	@
	ε-Poly-L-lysine + Dart	3.5 oz + 45 fl oz	@	@	@	@
	Nisin + EDTA + Zinc oxide	13.5 oz + 40 fl oz + 32 oz	@	@	@	@
	"New" Mycoshield	8 oz	@	@	@	@
	Cueva	96 fl oz	@	@	@	@
Rotation	Badge X2	16 oz	@	---	---	---
Rotation	Badge X2 + ProPhyt	16 oz + 64 fl oz	---	@	---	---
Rotation	Blossom Protect + Buffer	20 oz + 5 lb	---	---	@	---
Rotation	Blossom Protect + Buffer	20 oz + 5 lb	---	---	---	@
	Kasumin 4L	32 fl oz	@	@	@	@
	Kasumin 2L	64 fl oz	@	@	@	@
	Kasumin 2L + ε-Poly-L-lysine + Zinc oxide	64 fl oz + 13.5 + 32 oz	@	@	@	@
	Kasumin 2L + FireWall	64 fl oz + 8 oz	@	@	@	@
	Blossom Protect + Buffer	20 oz + 5 lb	@	@	@	@

All trees received a bud break treatment with 6 lb ChampION/A on 3-25-19. In-season treatments were applied on 3-30 (5-10% bloom), 4-4 (20-40% bloom), and 4-10-18 (full bloom), and 4-19 (petal fall) using an air-blast sprayer. Disease was evaluated on 5-21-19. "New" Mycoshield formulation = NUP 17010.

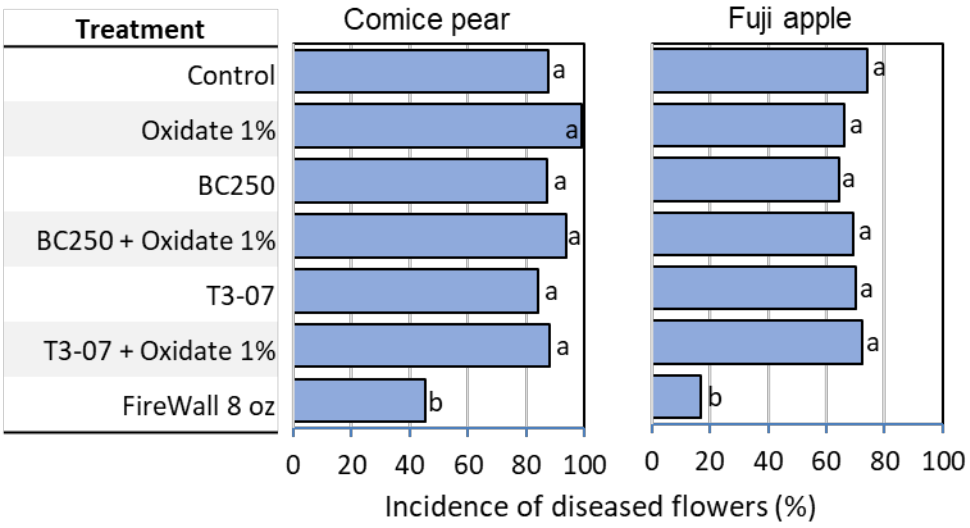
Fig. 3. Efficacy of bactericides for management of fire blight of Fuji apples, Fresno Co. 2019

Treatment	Rate/A
Control	---
Nisin + EDTA + Zinc Oxide	13.5 oz + 40 oz + 32 oz
ε-Poly-L-lysine + Dart	13.5 oz + 45 fl oz
ε-Poly-L-lysine + Zinc Oxide + EDTA	13.5 oz + 32 oz + 40 fl oz
ε-Poly-L-lysine + Zinc Oxide + EDTA	3.5 oz + 32 oz + 40 fl oz
Kasumin 4L	32 fl oz
FireLine + Zinc Oxide + Dart	16 oz + 32 oz + 45 fl oz
Kasumin 2L	64 fl oz



All trees received a bud break treatment with 6 lb ChampION/A on 3-25-19. In-season treatments were applied on 4-2 (5-10% bloom), 4-5 (20-40% bloom), 4-12-18 (full bloom), and 4-22 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated for 100 flower clusters (spurs) of each tree on 5-20-19. All treatments had four, paired-tree replications (total of 8 trees).

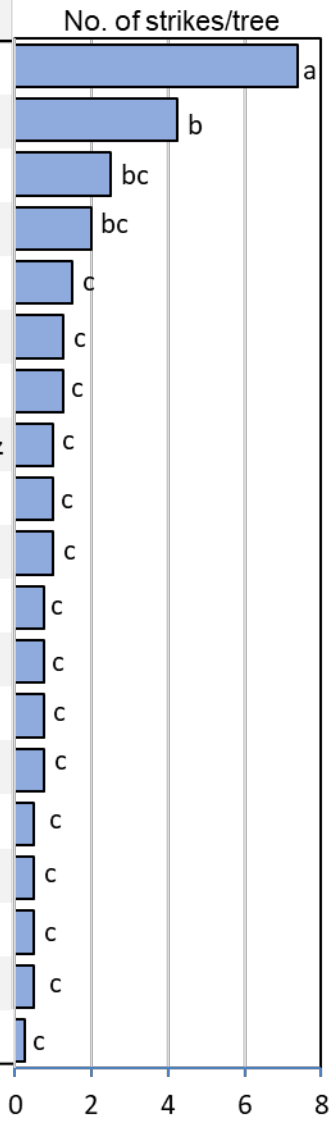
Fig. 4. Efficacy of two new potential bacterial antagonists (BC250, T3-07) in comparison with streptomycin for control of fire blight of Comice pear and Fuji Apple in a small-scale field study at UC Davis 2019



Treatments were applied to open flowers using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3.5 h, and Oxidate was applied after another hour. Disease was evaluated after 1 week.

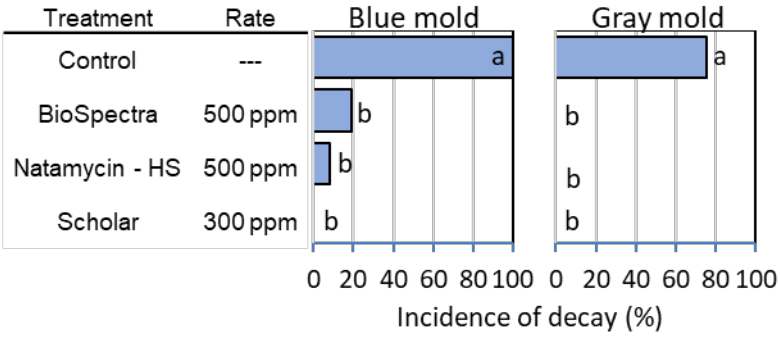
Fig. 5. Efficacy of new bactericides for management of fire blight of Bartlett pear, Sutter Co. 2019

Treatment	Rate
Control	---
Nisin + EDTA	13.5 oz 32 fl oz
NSA + Silco	135 fl oz + 68 fl oz
NS1 + Silco	135 fl oz + 68 fl oz
NS2 + Silco	135 fl oz + 68 fl oz
ε-Poly-L-lysine + ZnNO3	13.5 oz + 50 fl oz
New Mycoshield + ZnNO3 + LI700	8 + 50 fl oz + 8 fl oz
Kasumin + ε-Poly-L-lysine + ZnO	64 fl oz + 13.5 oz + 32 oz
New Mycoshield + LI700	8 oz + 8 fl oz
Cueva	96 fl oz
ε-Poly-L-lysine + DART	13.5 oz + 44 fl oz
Kasumin	64 fl oz
Kasumin + ε-Poly-L-lysine	64 fl oz + 13.5 oz
Blossom Protect + buffer	20 + 80 oz
FireLine + ZnO + LI700	16 oz + 32 oz + 8 fl oz
Serenade ASO + Cueva	64 + 96 fl oz
Nisin + EDTA + ZnO	13.5 + 32 + 32 oz
Kasumin 4L	32 fl oz
Kasumin + Firewall	64 fl oz + 8 oz



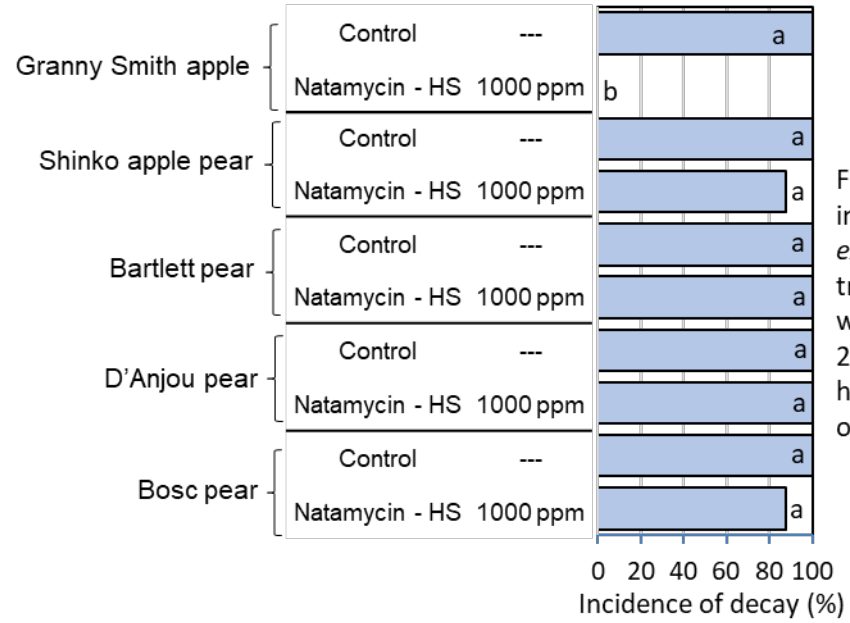
Treatments were applied on 4-4 (5% bloom), 4-12 (full bloom), 4-18 (petal fall), and 4-26-19 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated on 5-1-19.

Fig. 6. Evaluation of postharvest treatments with two formulations of natamycin for managing postharvest decays of Granny Smith apple in laboratory studies



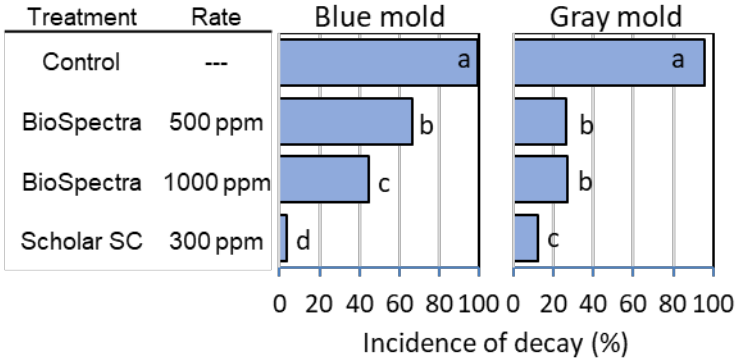
Fruit were wound-inoculated with *P. expansum* or *B. cinerea* and spray-treated after 12 h. Fruit were then incubated at 20C. Natamycin – HS = a high-solution formulation of natamycin.

Fig. 7. Evaluation of postharvest treatments with natamycin for managing postharvest blue mold of pome fruit cultivars in a laboratory study



Fruit were wound-inoculated with *P. expansum* and spray-treated after 12 h. Fruit were then incubated at 20C. Natamycin – HS = a high-solution formulation of natamycin.

Fig. 8. Evaluation of postharvest treatments with natamycin and Scholar for managing postharvest decays of Granny Smith apple in an experimental packingline study



Fruit were wound-inoculated with *P. expansum* or *B. cinerea* and treated by aqueous in-line drench applications with fungicides after 15-16 h. Fruit were sequentially treated with a CDA application with diluted carnauba wax. Fruit were then incubated at 20C for 7 days.


Appendix G