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.

Kasugamycin

Crops

Identification of Petitioned Substance

Chemical Names:
Kasugamycin
2-amino-2-[(2*R*,3*S*,5*S*,6*R*)-5-amino-2-methyl-6-[(2*R*,3*S*,5*S*,6*S*)-2,3,4,5,6-pentahydroxycyclohexyl]oxyoxan-3-yl]iminoacetic acid (IUPAC name)

3-O-[2-amino-4-[(carboxyiminomethyl)amino]-2,3,4,6-tetradeoxy-a-D-arabino-hexopyranosyl]-D-chiro-inositol

Other Name:
Kasugamycin hydrochloride hydrate
Kasugamycin monohydrochloride

Trade Names:
Kasumin 2L, Kasumin 4L

CAS Numbers:
Kasugamycin (6980-18-3)
Kasugamycin monohydrochloride (19408-46-9)

Other Codes:
Kasugamycin Pub Chem CID 65174

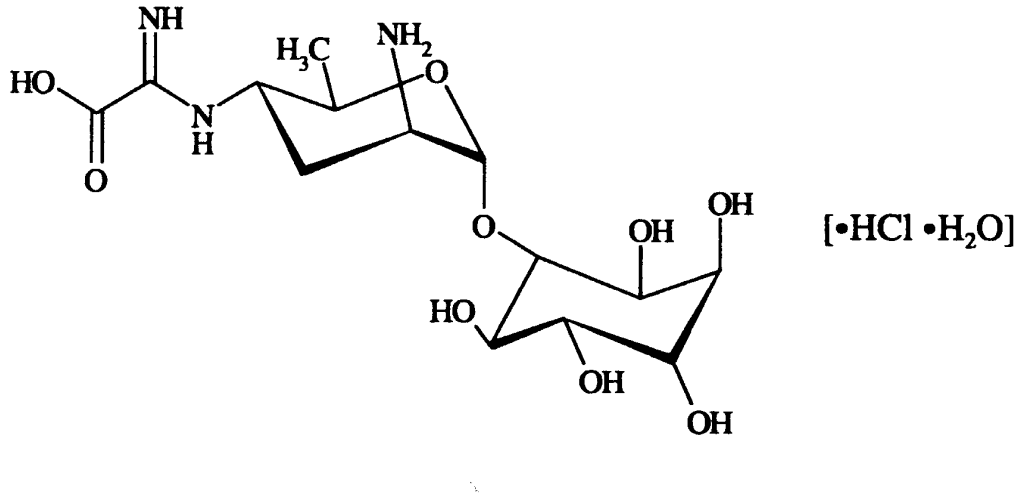
Summary of Petitioned Use

The National Organic Program (NOP) was petitioned to add kasugamycin as an allowed synthetic to the synthetic substances National List at 7 CFR §205.601. Alternate formulated names are kasugamycin monohydrochloride and kasugamycin hydrochloride hydrate. The specific petitioned use is for control of fire blight caused by *Erwinia amylovora* in apples, pears, and other pome fruits (California Apple Commission 2020).

Characterization of Petitioned Substance

Composition of the Substance:
Kasugamycin is an aminoglycoside containing the sugar inositol. Kasugamycin hydrochloride is isolated during the manufacture of kasugamycin. Kasugamycin hydrochloride hydrate is the registered active ingredient in the Kasumin brand formulations. The structure of kasugamycin hydrochloride hydrate is shown in Figure 1 (U.S. EPA 2005). The inositol moiety is on the right-hand side of the illustration.

Figure 1. Structure of Kasugamycin Hydrochloride Hydrate



Source or Origin of the Substance:

Kasugamycin is obtained by aerobic fermentation of the microorganism *Streptomyces kasugaensis*. This microorganism was originally discovered near the Kasuga Grand Shrine in Nara City, Japan. The active ingredient kasugamycin hydrochloride hydrate is isolated from kasugamycin fermentation product (Umezawa et al. 1967).

Properties of the Substance:

Kasugamycin is a colorless solid at room temperature and normal atmospheric pressure. The free base melts with decomposition at 214–216°C. The molecular formula is $C_{14}H_{25}N_3O_9$ and the molecular weight is 379.36 g/mol (U.S. EPA 2005; PubChem 2020a).

Kasugamycin hydrochloride is composed of white crystals that melt with decomposition at 236–239°C. The molecular formula is $C_{14}H_{26}ClN_3O_9$ and the molecular weight is 415.82 g/mol. The hydrochloride hydrate has a molecular weight of 433.8 g/mol. The bulk density of the hydrochloride hydrate is 0.43 g/ml at 24.5°C.

The hydrochloride is soluble in water with a maximum solubility of about 228 g/liter at pH 7. It is more soluble in alkaline solutions. The Environmental Protection Agency (EPA) reports 207 g/liter at pH 5, 228 g/liter at pH 7, and 438 g/liter at pH 11 (U.S. EPA 2005). Kasugamycin hydrochloride hydrate is the active ingredient in the Kasumin formulations (U.S. EPA 2018).

Kasugamycin hydrochloride hydrate is insoluble in ethanol, acetone, ethyl acetate, chloroform, and benzene and sparingly soluble in methanol. It has three ionizable groups, carboxyl group, cyclic primary amine, and secondary amine, with pKa values of pKa1 = 3.23, pKa2 = 7.73 and pKa3 = 11.0. The pKa1 refers to ionization of a carboxyl group. The pKa2 measures ionization of the cyclic primary amine. The pKa3 measures ionization of the secondary amine (U.S. EPA 2005).

When the hydrochloride is dissolved in water, the carboxyl group ionizes, making the solution acidic. Aqueous solutions (1 percent wt/vol) of the hydrochloride are acidic with a pH of 4.35 at 24.5°C. It is much more soluble in alkaline solutions. Raising the pH from 5 (207 g/liter) to 9 (438 g/liter) more than doubles solubility (U.S. EPA 2005). More alkaline solutions, however, tend to be unstable, and undergo slow decomposition. See *Evaluation Question #4* for more information.

Kasugamycin hydrochloride has relatively low volatility with a vapor pressure of <0.013 mPa at 25°C (about 0.13 atmospheres). Dried residues of the hydrochloride do not volatilize readily from soil into air. The hydrochloride is about 100 times more soluble in water than octanol with log Kow (i.e., the

concentration of the test substance in octanol divided by the concentration in water) = -1.96. PubChem (2020b) provides an estimate of log Kow = -5.75. The higher solubility of the hydrochloride in water versus octanol means that there is little tendency for bioaccumulation in aquatic organisms. See *Evaluation Question #6* for more information.

In water between pH 5–9, kasugamycin hydrochloride solutions form a zwitterion. This means the carboxylic group is mostly ionized, and the proton is captured by the primary amine. This salt-like ion is not very volatile, and kasugamycin does not volatilize readily from water (U.S. EPA 2013). See *Evaluation Question #4* for more information.

Specific Uses of the Substance:

The specific use described in the petition is for the control of fire blight caused by *Erwinia amylovora* in apples, pears, and other pome fruits. In a small-scale trial with Bartlett pears, kasugamycin at 100 ppm applied 18 hours after inoculation with *Erwinia* reduced disease incidence by more than 66 percent (Adaskaveg et al. 2009).

In commercial California pear orchards from 2006–2010, kasugamycin applied three to six times at 100 ppm during bloom led to a 77–78 percent reduction of fire blight disease incidence in fields deliberately inoculated with *Erwinia*. In fields not inoculated with *Erwinia*, there was an 80–90 percent reduction in disease incidence. Kasugamycin was more effective than oxytetracycline, and it worked equally well on isolates either resistant or not resistant to streptomycin. More than four applications led to phytotoxicity (Adaskaveg et al. 2009; Adaskaveg et al. 2011).

In *Erwinia*-inoculated Bartlett pear and Golden Delicious apple orchards in Oregon, two applications of kasugamycin at 100 ppm reduced disease incidence 93 percent in pears and 77 percent in apples (Johnson et al. 2008). In the most favorable case among Jonathan or Gala apples in New York orchards, 100 ppm kasugamycin was applied the day before and the day after inoculation with *Erwinia*. Disease control after application was 91 percent (Sundin 2014).

Kasugamycin has also been used to control other plant diseases that are described in *Historic Use*.

Approved Legal Uses of the Substance:

The U.S. EPA has registered Kasumin 2L and Kasumin 4L for control of plant diseases, especially fire blight caused by *Erwinia amylovora* on apples and pears. These are formulations containing the active ingredient kasugamycin hydrochloride hydrate (U.S. EPA 2018; U.S. EPA 2020).

Action of the Substance:

Kasugamycin is an antibiotic that inhibits bacterial protein synthesis. This process is discussed further in *Evaluation Question #5*.

Combinations of the Substance:

Kasugamycin is not a precursor to, a component of, nor used in combination with another substance on the National List. Currently registered formulations are Kasumin 2L and Kasumin 4L, which are not compliant with NOP requirements for organic production. These contain surfactants and the preservative 1,2-benzisothiazolone which are not allowed as inerts under 7 §CFR 205.601(m) (Kasumin SDS 2015; U.S. EPA 2018; U.S. EPA 2020;). The petitioner states that the manufacturer is willing to develop a formulation that is compliant for organic use if kasugamycin is approved (California Apple Commission 2020).

Status

Historic Use:

The Japanese scientist Hamao Umezawa and his colleagues produced kasugamycin by aerobic fermentation of *Streptomyces kasugaensis* in 1965 (Umezawa et al. 1965; Umezawa et al. 1967). Production with a different organism, *Streptomyces kasugaspinus*, was patented later (Umezawa et al. 1971).

Tamamura and Sato (1999) found "that kasugamycin possesses weak or almost no antibacterial activity against common pathogenic bacteria in human or animals." According to Levitan (1967), "kasugamycin was noted to be more effective against *Pseudomonas* species than against some of the other bacteria tested, results were nevertheless uniformly disappointing" (753).

The earliest application in agriculture was in 1965 as an antibiotic for the pathogen *Piricularia oryzae*, which causes rice blast disease (Masukawa et al. 1968; Umezawa et al. 1974). Kasugamycin is used in Mexico to control bacterial rot, *Erwinia atroseptica*, and leaf mold, *Cladosporum fulvum*, on tomato. It is also used to control bacterial spot caused by *Xanthomonas campestris pv. vesicatoria* on tomato and pepper (U.S. EPA 2005).

The EPA established tolerances for kasugamycin on August 29, 2014 of 0.20 ppm for pome fruit (Fed Reg 2014). Current tolerances for kasugamycin set by the FDA at 21 §CFR 180.614 are 0.04 ppm for fruiting vegetables and 0.20 ppm for pome fruit.

The technical grade active ingredient, kasugamycin hydrochloride hydrate, was registered with the EPA on September 8, 2014 with the registration number 66330-403 (U.S. EPA 2014). The formulation Kasumin 2L containing two percent kasugamycin was registered March 1, 2018 with registration number 66330-404 (U.S. EPA 2018). Kasumin 4L containing four percent kasugamycin was registered January 15, 2020 with the registration number 66330-436 (U.S. EPA 2020).

Kasumin 4L and Kasumin 2L were registered with a number of restrictions including those that prohibit application where animals are grazing or in areas where crops have been fertilized with animal or human waste. Users are also required to follow a resistance management plan. Applications are limited to four per year with the sole exception of California, where the limit is two applications per year (U.S. EPA 2020).

Kasumin registration was for diseases of cherry, pome fruit and walnuts. Diseases of cherry included bacterial blast and bacterial canker caused by *Pseudomonas syringae pv. syringae*. For cherry, the preharvest interval is 30 days. It was registered for fire blight, *Erwinia amylovora*, on pome fruit including apple and pear. For fire blight, there is a 90-day preharvest interval. For walnut blight, caused by *Xanthomonas campestris pv. juglandis*, the preharvest interval is 100 days (U.S. EPA 2020).

Kasumin 2L containing the active ingredient kasugamycin hydrochloride hydrate was registered in California on January 1, 2018 for diseases of almond, apple, cherry, pear, and walnut (CA DPR 2020).

Organic Foods Production Act, USDA Final Rule:

Kasugamycin is not mentioned in the Organic Foods Production Act (OFPA), nor is it listed at 7 CFR §205.601, synthetic materials allowed for organic crop production. It is also not listed at 7 CFR §205.603 for livestock production, nor at 7 CFR §205.605 for processing.

International

Canada – CAN/CGSB-32.311-2020, Organic Production Systems, Permitted Substances Lists

Kasugamycin is not listed in Table 4.2, Substances for Crop Production, nor in the alphabetized list of materials (Canada 2020).

http://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-311-2020-eng.pdf

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

Kasugamycin is not listed in the Codex Alimentarius of Organically Produced Foods.

http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

http://www.codexalimentarius.org/download/standards/360/cxg_032e.pdf

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008

Kasugamycin is not listed in European Community Council Regulation No. 834/2007 (ECC 2007).

Kasugamycin is not listed in European Community Council Regulation No. 889/2008. Specifically, it is not listed in Annex II, pesticides-plant protection products, referred to in Article 5(1). It is also not listed in Annex VIII, certain products and substances for use in processed organic food, referred to in Article 27(1)(a) (ECC 2008).
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:250:0001:0084:EN:PDF

Japan Agricultural Standard (JAS) for Organic Production
Kasugamycin is not listed in the Japanese Agricultural Standard for Organic Plants. It is specifically not listed in Table 2, Substances for Plant Pest and Disease Control, nor in Table 4 Chemical Agents (Japan 2017).
http://www.maff.go.jp/e/jas/specific/criteria_o.html
http://www.maff.go.jp/e/policies/standard/jas/specific/criteria_o.html

International Federation of Organic Agriculture Movements (IFOAM) – Organics International Norms
Kasugamycin is not listed in Appendix 3, Crop Protectants and Growth Regulators (IFOAM 2014).
http://www.ifoam.bio/en/ifoam-norms

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

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

Kasugamycin is a bacterial toxin produced by *Streptomyces kasugaensis*. It is not a synthetic inert ingredient (Umezawa et al. 1967; California Apple Commission 2020).

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

Kasugamycin is manufactured by aerobic fermentation of *Streptomyces kasugaensis*. In a typical fermentation, after inoculation with the microorganism, 100 liters of sterilized growth medium is fermented for 48 hours at 28°C at about pH 7.4. The solution is aerated at 100 liters/minute and agitated at 200 rpm. This solution is then added to 1,400 liters of the same growth medium. After 90 hours, the pH is 7.2 and 530 micrograms/ml of kasugamycin is produced (Umezawa et al. 1967).

To isolate the product, solids in the fermentation medium are removed by centrifugation or filtration. The pH is adjusted to 7.0 and the liquid is treated with activated charcoal to remove impurities. The charcoal is extracted with butanol and water to remove impurities, then kasugamycin is eluted as the hydrochloride from the charcoal with pH 2 hydrochloric acid solution. This solution is freeze dried to a crude powder containing kasugamycin hydrochloride (Umezawa et al. 1967; California Apple Commission 2020).

An aqueous solution of the powder is applied to a column of cationic ion exchange resins (IRC-50) to remove basic impurities. The aqueous effluent containing kasugamycin hydrochloride is added to a column of IRC-120 resin where it is adsorbed. It can be eluted with aqueous ammonia at cold temperatures

(15°C), and the effluent is subsequently neutralized with HCl to pH 6.6. The eluate is concentrated to dryness or freeze dried to obtain kasugamycin hydrochloride as a crude powder. The eluate can also be concentrated in a vacuum, and kasugamycin hydrochloride of 90 percent purity can be obtained as crystals by the addition of ethanol (Umezawa et al. 1967).

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

Kasugamycin is created by a naturally occurring biological process, the fermentation of *Streptomyces kasugaensis* (Umezawa et al. 1967). The extraction and purification of kasugamycin from the fermentation broth involves elution with hydrochloric acid, yielding the salt form of crystalline kasugamycin hydrochloride. Although the process for manufacturing kasugamycin is biological, kasugamycin is chemically isolated as the hydrochloride. This chemical change is not created by a naturally occurring biological process or created through heating or burning biologic matter. Questions of chemical change, and processes used to create that change, are part of the criteria used in NOP Guidance 5033-1 *Classification of Materials as Synthetic or Nonsynthetic* (USDA NOP 2016).

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

Kasugamycin is characterized as moderately persistent to persistent (U.S. EPA 2013). A major source of degradation is aerobic microbial metabolism in soil with a half-life of 43-73 days. About four percent remains after a year. Because laboratory studies used only one soil type, the EPA uses a 219-day soil half-life in persistence calculations (U.S. EPA 2013).

Both aerobic and anaerobic degradation occurs. Aerobic degradation is faster than anaerobic. Typical aerobic half-life in water is seven days and half-life in sediment is 108 days. Anaerobic half-life was 32 days in water and 141 days in sediment (NYS 2015; U.S. EPA 2013).

Hydrolysis in water is very slow, especially in acidic conditions (NYS 2015). Kasugamycin moves freely in sandy soil, less so in clay soils. It is likely to move both into surface water and ground water, but movement into ground water is less likely (U.S. EPA 2013). Because of soil movement, field dissipation is faster than molecular degradation seen in the laboratory. Field dissipation half-life in soil is 5.7 to 12.3 days. It does not volatilize readily from water or soil. Half-life of kasugamycin in the gas phase is 1.6 hours (NYS 2015).

The major metabolites are kasugamycinic acid (CAS No. 6001-03-2) and kasuganobiosamine (CAS No. 6189-93-1). Kasugamycinic acid (CAS No. 6001-03-2) results from conversion of the imino group of kasugamycin to a carboxylic acid. Kasuganobiosamine (CAS No. 6189-93-1) results from loss of the acetic acid amide group leaving the free amine. Kasuganobiosamine has two free amino groups (U.S. EPA 2013).

These metabolites are also persistent. About 3.1 percent of the acid was left after 180 days in aerobic/anaerobic rice paddy systems. About 28.7 percent was left after about a year in anaerobic aquatic laboratory studies. About 44.7 percent of the amine was left after a year in the anaerobic aquatic studies. The amine was destroyed quickly by aqueous photolysis (3.2 percent left after 18.9 days); the acid metabolite was more persistent (48.5 percent left after 18 days (U.S. EPA 2013). Details on degradation are provided below.

Mobility in Soil

Kasugamycin may be less persistent in the field than laboratory experiments suggest. Field dissipation is more rapid, with a soil half-life ranging from 5.7–12.3 days. Kasugamycin did not leach in eastern soil below 15 cm (6 in); in California soil, leachate traveled less than 30 cm (12 in) (NYS 2015; U.S. EPA 2013). The big difference between laboratory persistence (43–75 days half-life) and field persistence (12.3 days) is that field measurements include movement from the application site, whereas lab experiments are measuring degradation of the molecule.

Kasugamycin moves freely in soil, but there is a range depending on the soil type. The K_{oc} (soil adsorption coefficient) for sandy soil was 10 ml/g; for clay loam, 364 ml/gram. The larger the K_{oc}, the stronger binding to soil. Up to five percent of applied amounts could run off into surface water (NYS 2015).

Using a solubility of 228 g/liter, an application rate of 0.336 lbs/acre/year, an absorption coefficient K_{oc} of 345 ml/g, and a half-life of 73 days, modeling experiments predicted the maximum amount leached from soil as 0.038 ppb. According to the modeling experiment, kasugamycin is not a likely ground water threat (NYS 2015). However, the metabolites range from moderate to highly mobile, and have the potential to be found in drinking water (U.S. EPA 2013).

The EPA (2012) estimates that the acute drinking water concentration of kasugamycin is 0.011562 ppm, the chronic drinking water concentration is 0.00178, and the groundwater concentration is 0.000116 ppm.

Persistence in Water

In laboratory experiments, kasugamycin degrades in water and sediment by both aerobic and anaerobic processes. The aerobic half-life in water is 6.9 days, and the aerobic half-life in sediment is 108 days. In anaerobic systems, the half-life in water was 32 days and in sediment, 141 days (NYS 2015).

In one experiment, kasugamycin showed an anaerobic aquatic half-life of 105 days. Metabolites were kasugamycinic acid and kasuganobiosamine (NYS 2015). In another experiment, aerobic aquatic half-life ranged from 103 to 147.5 days. Kasugamycinic acid was the major species (NYS 2015).

Kasugamycin slowly degrades by hydrolysis in water. Half-life in acidic conditions ranges from 462–630 days. At pH 7, half-life is 80 days, and under alkaline conditions (i.e., pH 11) it is 11.4 days (NYS 2015).

Bioaccumulation in aquatic organisms is low. Kasugamycin is much more soluble in water than octanol (PubChem 2020a; U.S. EPA 2005).

Persistence in Air

Kasugamycin has a low vapor pressure and is not volatile from soil surfaces. In the air, kasugamycin exists both in gaseous and particulate phases. Photochemical half-life in the gas phase is 1.6 hours (PubChem 2020a).

Kasugamycin is not expected to volatilize from water because it is a zwitterion from pH 5–9. This means the carboxylic acid is extensively ionized, and the hydrogen ion forms a salt with the amine group of kasugamycin (PubChem 2020a).

Persistence on Fruit

About half the amount applied to foliage ends up on the soil and non-target surface vegetation. Residues on fruit decrease 10-fold in 27–32 days. This fact means that with a 90-day preharvest interval, residues at harvest time on fruit are 1/1000 of that originally applied (NYS 2015; PubChem 2020a).

The NOSB Crops Subcommittee is interested in possible kasugamycin residues on fruit, and posed this question:

How does timing affect potential for residue in fruit at harvest? And are there any residues in fruit at harvest?

Kasugamycin is applied for fire blight on apples and pears during bloom. There is a 90-day preharvest interval. According to PubChem (2020a), there is a 10-fold decrease in residues every 27–32 days. From the PubChem data, residues in 90 days are roughly 1/1000 of that applied (PubChem 2020a). The application rate is 100 ppm (parts per million), and the residues at harvest should be about 0.1 ppm. The detectable limit of kasugamycin in apples is about 6 microgram/kg or 6 ppb. The maximum residues allowed, or tolerance, is 0.2 ppm. Measured residues at harvest should be between 6 ppb (parts per billion) and 0.2 ppm (Fed Reg 2014; Wang et al. 2017).

Much of the actual residue information for kasugamycin is unpublished proprietary information (U.S. EPA 2005). Kasugamycin is not currently included in the USDA Pesticide Data Program residue monitoring database (USDA 2019). A search of PubChem on Sept 22, 2020 for kasugamycin residues on apples returned no results. Residues can be estimated from tolerances (Fed Reg 2014) and calculated exponential decay of applied dose with time, as shown above (PubChem 2020a).

The maximum residue in apples and pears allowed at harvest is 0.2 ppm, or 0.2 mg/kg (Fed Reg 2014). Likely consumption is no more than a pound of apples or pears a day, or 0.5 kg/day. A pound of apples would contain a maximum 0.1 mg of kasugamycin. If a 10–kg (22 lb) child ate a pound of apples, exposure would be 0.01 mg/kg body weight. The EPA Reference Dose is 0.113 mg/kg/day (NYS 2015). Consumption of less than this amount is presumed to cause no problems. A likely worst-case exposure would be about 1/10 the reference dose if the residues equaled tolerance levels.

The application rate of kasugamycin to apples and pears is 100 ppm. From PubChem data, residues are likely 1/1000 of that in 90 days. Residues should be no more than 0.1 ppm at 90 days. This is one half the tolerance of 0.2 ppm. A likely worst-case consumption in the case of 10-kg child would be about 1/20th the reference dose (PubChem 2020a).

Key to residue levels is the time between the last spray and harvest. The minimum time between the last kasugamycin application and harvest is 90 days, but according to the variety, fruit can be harvested more than 90 days after the last spray. No kasugamycin sprays can be applied after petal fall and fruit set (U.S. EPA 2018). Varieties such as Fuji and Granny Smith that take longer to mature after fruit set should have fewer residues than Gala or Gravenstein that mature closer to the date of the last spray.

Streptomycin, another fire blight control in apple, pear, and other pome fruit production, is also applied at 100 ppm. In one study the highest concentration of streptomycin found on apples after three sprays was 0.018 mg/kg of fruit. The highest concentration of residues was in the apple core (Stockwell 2014). If similar residues are found with kasugamycin, this is 0.018 ppm or 0.02 ppm. If a 10-kg child ate a pound of apples, exposure would be 0.001 mg/kg body weight, about 1/100 of the reference dose.

Residues at harvest should be between 0.02 and 0.20 ppm, and result in worst case exposures between 1/10 and 1/100 of the reference dose. The lowest detectable residue would be 6 ppb.

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

Toxicity of Kasugamycin

Kasugamycin has low acute toxicity to mammals. The oral median lethal dose (LD50) (i.e., the amount that causes death in 50 percent of test animals; a low number indicates high toxicity) in rats is >5000 mg/kg. Similar oral toxicity was seen in mice. The acute dermal toxicity in rats is >2000 mg/kg. Kasugamycin is a mild eye irritant but is not irritating to the skin. It is also not a skin sensitizer. However, the Kasumin formulation is a sensitizer, and may trigger allergies if exposed; see *Evaluation Question #10*. Kasugamycin is classified EPA Category IV (least toxic, no warning on label) for all exposures other than dermal, for which it is Category III (next least toxic, requires "Caution" warning on label) (U.S. EPA 2005). The National Library of Medicine database PubChem lists the oral LD50 in rats as 11,400 mg/kg; the dermal LD50 is >4,000 mg/kg. Mouse oral is 21,000 mg/kg and dermal is >10,000 mg/kg (PubChem 2020a). In rats, only five percent of an oral dose is absorbed (PubChem 2020a).

Kasugamycin also has low chronic toxicity. In 90-day rat chronic feeding studies, the kasugamycin no-observed-adverse-effect level (NOAEL) is 176.7 mg/kg/day for males and 201.0 for females. These are relatively large doses. Adverse effects seen above these levels were decreased body weights and decreased weight gains (U.S. EPA 2005). In 90-day chronic feeding studies of mice, the kasugamycin NOAEL was 135.4 mg/kg/day for males and 170.9 for females. Based on increased mortality, anal lesions, and kidney

lesions, the lowest observed adverse effect level (LOAEL) for mice was 408.5 mg/kg/day for males and 565.6 for females. Again, these are relatively large doses (U.S. EPA 2005).

Kasugamycin was more toxic to dogs. In 90-day oral feeding tests, the NOAEL was 10.6 mg/kg/day for males and 11.4 for females. Based on tongue lesions, fewer feces, swollen mouth, excessive salivation, and thickened skin in the mouth, LOAEL was 106 mg/kg/day for males and 107.9 for females (U.S. EPA 2005).

Reproductive and developmental effects were seen in rats at high doses. Prenatal studies in rats found the maternal NOAEL was 200 mg/kg/day. Based on decreased body weights, the LOAEL was 1000 mg/kg/day. The LOAEL for developmental effects in offspring was >1000 mg/kg/day. Rabbits were more sensitive. The maternal LOAEL based on spontaneous abortions and reduced body weight was >10 mg/kg/day (U.S. EPA 2005).

Based on decreased fertility and fecundity in first generation parents and an increased pre-coital interval during the mating period for the second generation, reproductive toxicity LOAEL was 425.3 mg/kg/day for male rats and 503.4 for females (U.S. EPA 2005).

No evidence of carcinogenicity was seen in mice at a NOAEL of 186.3 mg/kg/day for males and 215.2 for females. No evidence of carcinogenicity was seen in rats at a NOAEL of 11.3 mg/kg/day for males and 140 for females. Increased testicular softening and atrophy of testicular tubules was seen in males at those doses (U.S. EPA 2005).

Kasugamycin is not mutagenic and shows no evidence of chromosome damage (U.S. EPA 2005). The EPA classifies kasugamycin as "not likely to be carcinogenic in humans" (U.S. EPA 2013)

In rats, more than 90 percent of a dose is excreted within 168 hours. Most (82–94 percent) is excreted in the feces. In rats, <5 percent of a dose was metabolized—most was excreted unchanged. Maximum blood concentrations were seen within one hour of an oral dose (U.S. EPA 2005). With an intramuscular injection of 1 g into humans, 63 percent was excreted unchanged in urine within eight hours. In an oral administration to mice of 100 mg/kg, 43–68 percent was excreted in urine within six hours. In subcutaneous injections of 100 mg/kg into rabbits, 96 percent was excreted unchanged in urine after eight hours (PubChem 2020a).

Mode of Action

Kasugamycin interferes with bacterial protein synthesis. Proteins are synthesized in the ribosome. During protein synthesis, DNA is transcribed into messenger RNA (mRNA) that travels to the ribosome, interacting there with transfer RNA (tRNA). mRNA contains codons that interact with tRNA anticodons, telling tRNA which amino acids to add to the growing peptide chain (Culver 2001).

Kasugamycin interferes with tRNA binding in the ribosome. Specifically, it inhibits "initiation of translation by blocking initiator tRNA binding to the 30S subunit" (Schuwirth et al. 2006). Kasugamycin binds to the 30S ribosome "in the region of the mRNA binding tunnel in the E-site and P-site and indirectly inhibits tRNA binding at the P-site by perturbing the mRNA-tRNA codon-anticodon interaction during translational initiation" (Yoshii et al. 2012). Basically, kasugamycin blocks movement of mRNA through the ribosome, preventing effective interaction with tRNA.

Persistence in the Environment

See *Evaluation Question #4* for more information.

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

Manufacturing of the petitioned substance involves only mineral salts, organic feedstocks such as maltose or corn steep liquor, acids, bases, and ion exchange resins. Ion exchange resins can be recycled. Other items have low toxicity, and the quantities involved should not cause an environmental impact. Little of the

waste is hazardous—see *Evaluation Question #2* for more information. Effects of use and misuse are covered in *Evaluation Question #8*. Disposal of kasugamycin is done according to labeled instructions for use as a pest control product.

Most of the environmental contamination from sprays is found on soil, soil vegetation, and in water. About 41–70 percent of spray applications are lost to the environment through runoff from vegetation and pesticide drift. About half of amounts applied to foliage end up on the soil and vegetation below the trees (NYS 2015). The active ingredient kasugamycin hydrochloride is water soluble and mobile in soil and can travel to water. Up to five percent could end up in surface water. When kasugamycin reaches surface water, most of it stays in the water; very little is bound to sediment (Huang et al. 2010; NYS 2015).

Analysis of irrigation water in rice paddies where kasugamycin was applied showed kasugamycin water contamination of <2 ppm (Sheu et al. 2010).

In one experiment, river water microcosms containing sediment were treated with kasugamycin at 168.7 mg/liter (700 times the field application rate) and 1462.9 mg/liter (6,000 times the field application rate). The pH was 8.1—note that kasugamycin degrades more quickly in alkaline solutions. After 30 days, 34.1 percent of kasugamycin had degraded at the low application rate. The higher concentration saw only 12.1 percent degradation in 30 days (Huang et al. 2010). The researchers also found the microbial spectrum in water was affected by kasugamycin. See *Evaluation Question #8* for more information.

Other researchers found the half-life in water was about seven days and the half-life in sediment was 108 days (NYS 2015; U.S. EPA 2013). See *Evaluation Question #4* for more information on kasugamycin persistence.

There is very little air pollution after aerosols from the spray settle out. Kasugamycin hydrochloride in the formulation is not volatile when residues dry on surfaces, forming a salt. Kasugamycin hydrochloride does not volatilize appreciably from water. See *Evaluation Question #4* for more information.

Kasugamycin hydrochloride is much more soluble in water than octanol and is not expected to bioaccumulate in aquatic organisms. See *Evaluation Question #4* for more information.

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

Kasugamycin solutions are acidic and will interact with any alkaline plant protection formulations. Kasugamycin and its solutions are incompatible with alkaline tank mixes (U.S. EPA 2005; U.S. EPA 2018). Since lime sulfur sprays are alkaline, kasugamycin sprays would interact. As lime sulfur is used early in the blooming period for blossom thinning, kasugamycin could be applied later to avoid interference (Johnson and Temple 2013).

Kasugamycin is an antibiotic and might interfere with some bacterial biocontrol agents (i.e., bacteria used to control plant pathogens). In integrated programs, kasugamycin is applied later in the bloom period to prevent killing bacterial biocontrol agents (Stockwell et al. 2008; Johnson et al. 2008). See *Evaluation Question #11* for more information.

Fixed coppers are applied in the dormant and early prebloom period to prevent fire blight spreading from overwintering cankers. Fixed coppers are not phytotoxic because copper hydroxide and other fixed coppers have low solubility at neutral pH (Dupont 2019). However, kasugamycin sprays are acidic and could cause phytotoxicity due to release of copper ions. Interaction would be minimized if kasugamycin were applied later in the blooming period.

Copper is generally compatible with yeasts but not with bacterial biocontrols. Potential phytoxicity is also an issue when copper is used simultaneously with an acidic buffer, such as one needed in Blossom

Protect™ biocontrol (Adaskaveg et al. 2019b; Dupont 2019). See *Evaluation Question #11* for more information. .

Insecticides that might be used with organic apples and pears include soap, oil, spinosad, neem oil, *Bacillus thuringiensis* (BT), codling moth virus, pheromones, kaolin, and natural pyrethrins. Soap, horticultural oil, neem oil, and kaolin are not generally applied to blossoms, as "[m]ost insecticides should not be applied during bloom" (Pfeiffer 2017). Kasugamycin sprays, however, are applied to blossoms—see *Evaluation Question #11*—and should therefore not interfere with these insecticides. BT is a formulation of protein crystals and spores that contains no living microbes, so kasugamycin should not interfere. The spinosad label makes no mention of chemical interactions with kasugamycin (U.S. EPA 2020b).

Organic fungicides include oil, soap, induced systemic materials (i.e., materials that induce systemic resistance in plants against pathogens), and microbials. Again, oil and soap are not generally applied to blooms. Microbial interference was discussed above (Pfeiffer 2017).

Induced systemic materials for fire blight are usually applied as a trunk paint, tree injection, or a foliar spray (Acimovic et al. 2017b). Potential for these materials' interaction with kasugamycin is low due to the latter's application on foliage. The induced systemic material acibenzolar-S-methyl (ASM) is compatible with streptomycin and increases its efficacy (Maxon-Stein et al. 2002).

Kasugamycin is formulated to kill bacteria. Bacterial antibiotics should have no effect on viruses; thus, codling moth virus should be compatible unless it is destroyed by acidic solutions. Codling moth virus used on pear and apple is usually applied after bloom (Pfeiffer 2017).

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

When kasugamycin is sprayed on fruit trees, about half of it ends up on soil and soil vegetation. Some of it leaves the site through pesticide drift, and up to five percent ends up in surface water (NYS 2015). As a result, the EPA Chronic Risk Quotient is exceeded for mammals that graze on grass and forbs or consume terrestrial invertebrates in the immediate area (U.S. EPA 2013). See *Evaluation Question #4* for more information.

Livestock should not graze on grass exposed to kasugamycin pesticide drift, as it could change their intestinal biome similar to changes found after exposure to streptomycin. Kasugamycin has not been evaluated, but spraying orchard grass with streptomycin at concentration levels used for fire blight led to an increase in antibiotic-resistant human pathogens found in sheep grazing on sprayed grass. *E. coli* resistant to streptomycin, ampicillin, tetracycline, and other antibiotics was found in sheep feces. Streptomycin resistant *Staphyloccus* was found in sheep nasal cavities (Scherer et al. 2013). To prevent antibiotic resistant pathogens developing from kasugamycin sprays, the Kasumin label bans animal grazing in treated orchards (U.S. EPA 2018). See *Evaluation Question #10* for more information.

Kasugamycin-resistant epiphytic bacteria were found in orchards treated with kasugamycin. McGhee and Sundin (2011) found kasugamycin resistance in 401 bacterial isolates from apple flowers, leaves, and soil samples in treated orchards. Tancos et al. (2017) were not able to find kasugamycin resistant epiphytic organisms in New York apples sprayed up to ten times, but the antibiotic changed the microbial spectrum in the orchard. See below.

Huang et al. (2010) studied kasugamycin treatments in river water microcosms containing sediment (as reference in *Evaluation Question 6*) and found that the microbial spectrum in the water was affected. Some bacteria in the microcosm were resistant to kasugamycin, and populations increased. Others were more susceptible, and populations decreased.

Kasugamycin is phytotoxic to apples and pears. Plant damage is seen with more than four applications of kasugamycin per year (Adaskaveg et al. 2011).

The NOSB Crops Subcommittee is interested in microbial resistance to kasugamycin:
Is the product susceptible to development of resistance with normal (labeled) use?

Normal labeled use of kasugamycin has led to field resistance in several pathogens. Kasugamycin was first used to control diseases of rice in Japan starting in 1965 with rice blast caused by *Magnaporthe grisea* (*Pyricularia oryzae*). Field resistance of rice blast was noticed in 1971. Kasugamycin was also used for rice bacterial grain and seedling rot caused by *Burkholderia glumae* and for rice bacterial brown stripe caused by *Acidovorax avenae* subsp. *avenae*. Field resistance to *Acidovorax* sp. occurred in 1990. Field resistance to *B. glumae* was observed in 2001 (Yoshii et al. 2012).

In Florida, rapid field resistance to kasugamycin was seen with bacterial spot of tomato caused by *Xanthomonas perforans* (Vallad et al. 2010).

In orchards that had been treated at least once with kusugamycin, McGhee and Sundin (2011) were able to find resistant bacteria in 401 field isolates from apple flowers and leaves and orchard soil samples. The authors stated, "Although we have not established the presence of a transferrable Ks^R gene [kasugamycin resistance gene] in orchard bacteria, the frequency, number of species, and presence of Ks^R enterobacterial species in orchard samples suggests the possible role of nontarget bacteria in the future transfer of a Ks^R gene to *E. amylovora*."

On the other hand, Tancos et al. (2017) were not able to find kasugamycin-resistant epiphytic microbes in New York apple orchards sprayed up to ten times. However, kasugamycin reduced the total numbers of bacterial epiphytes and changed the microbial distribution in the orchard. There were larger numbers of *Pantoea* sp. and smaller numbers of *Pseudomonas* sp. The authors found increased numbers of *Pantoea* sp. concerning because *Erwinia* streptomycin resistance likely originated on transposon Tn5393 of *Pantoea* sp. They questioned whether *P. agglomerans*—"the predominant epiphytic bacteria following kasugamycin application"—could provide resistance genes against streptomycin or, potentially, kasugamycin (Tancos et al. 2017).

Tancos et al. (2017) did not check for kasugamycin-resistant soil samples. But McGhee and Sundin (2011) found kasugamycin resistant soil bacteria in Michigan orchards. Kasugamycin-resistant epiphytic and soil bacteria provide a reservoir of resistant bacteria and could provide a pathway for horizontal transmission of resistance to *Erwinia*.

Field resistance of *Erwinia* to kasugamycin has not been seen with normal, labeled use, but kasugamycin was only registered for fire blight in 2014, and it was only registered in California in 2018. The Kasumin formulation was first registered with the EPA in 2018. The EPA considers resistance a possibility, and resistance management schemes are required by the label (U.S. EPA 2018).

Erwinia resistance to kasugamycin has been generated in the laboratory. Antibiotics must be transported inside a bacterial cell to kill it. *Erwinia* has two separate genes, *dpp* and *opp*, that produce proteins dipeptide permease (Dpp) and oligopeptide permease (Opp) that transport kasugamycin into the cell. *Erwinia* resistance to kasugamycin occurred in the laboratory when either one or both of these genes were altered by mutation (Ge et al. 2018).

McGhee and Sundin (2011) were able to produce kasugamycin resistance to *Erwinia* in the laboratory. Mutation of the ksgA gene led to *Erwinia* resistant mutants with reduced fitness due to slower growth rate and reduced virulence to pears.

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

When kasugamycin is sprayed in orchards, about half the amount applied ends up on the soil or non-target vegetation near the trees (NYS 2015; U.S. EPA 2013). For use on pome fruit, the EPA Chronic Risk Quotient is exceeded for mammals of all sizes that eat short grass in kasugamycin-treated orchards. The Chronic Risk Quotient is also exceeded for 15g mammals that eat short grass, broadleaf plants, and insects. Similar risks are seen for 35g mammals that eat broadleaf plants and insects (NYS 2015; U.S. EPA 2013). Risks are exceeded with small mammals. Presumably, there is some risk for larger animals, as the Kasumin label states, "animal grazing in treated areas is prohibited" (U.S. EPA 2018).

Kasugamycin is practically non-toxic to non-target terrestrial invertebrates. Acute toxicity to fish, aquatic invertebrates, birds, and mammals is very low. Chronic feeding experiments in birds (NOAEC 450 mg/kg) led to reduced 14-day survival. In mammals (NOAEL 13.7 mg/kg body wt.), the chronic feeding experiments led to reduced body weight and reduced weight gains (U.S. EPA 2013).

The risk to terrestrial plants is uncertain due to lack of data. Risk to the environment from reduction of microbial populations or changes in microbial distribution are unknown and uncertain (U.S. EPA 2013). But antibiotic resistance to kasugamycin has been seen in orchard microbials (McGhee and Sundin 2011); see *Evaluation Question #10* for more information.

Kasugamycin is not expected to pose risks to wild mammals, birds, earthworms, honey bees and aquatic organisms at the proposed use rates. Because of a risk to plants, a buffer zone is required in Canada to minimize potential for exposure to off-field drift (Canada 2012). A similar buffer zone is required in the U.S. (U.S. EPA 2013).

None of the dicots tested by the EPA had risk quotients that exceeded EPA guidelines. Monocots such as onion and wheat had reduced dry weights when exposed to the test concentration of 0.0925 mg ai/acre (U.S. EPA 2013).

Kasugamycin has low acute toxicity to birds. The oral LD50 for kasugamycin in Japanese quail is >4,000 mg/kg. For the bobwhite quail, *Colinus virginianus*, the number is >2,000 mg/kg (PubChem 2020a). For zebra finch, the oral LD50 is >2000 mg/kg. For mallard duck, the LD50 is >2000 mg/kg; below this value, loss of body weight was observed. In chronic five-day feeding, the LC50 for mallard duck was >4,858 ppm. This concentration is classified as slightly toxic. Body weight changes were noticed at 581 ppm. These concentrations are ten times those expected from application at label rates (NYS 2015; U.S. EPA 2013).

The LD50 for acute contact toxicity in the honey bee is >100 microgram (mcg)/bee. The acute oral LD50 is 30.3 mcg/bee. For comparison, the oral LD50 for neonicotinoids is 3-5 ng/bee, and neonicotinoids are about 10,000 times more toxic. There is low kasugamycin toxicity to earthworms, as the EC50 is >1,000 mg/kg (U.S. EPA 2013).

Water Contamination

The Kasumin label states, "Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark" (U.S. EPA 2013).

Up to five percent of applied amounts of kasugamycin move into in surface water. Kasugamycin had the largest harmful effect on aquatic plants, especially blue-green algae. For duckweed, *Lemna gibba*, frond count was reduced with EC50 = 86 ppm. For green algae, *Pseudokirchneriella subcapitata*, 96-hour cell density was reduced with EC50 of 3.9 ppm. For blue-green algae, *Anabaena flos-aquae*, 96-hour cell density was reduced with EC50 of 0.65 ppm (NYS 2015). The most sensitive plant tested was blue-green algae, *Anabaena* sp., with EC50 0.65 ppm and a no-observed-adverse-effect concentration (NOAEC) of 0.08 ppm (U.S. EPA 2013).

Kasugamycin water contamination measured in rice paddy irrigation water was <2 ppm (Sheu et al. 2010). Huang et al. (2010) noted bacterial population changes when adding kasugamycin at high rates to river water microcosms in the laboratory—see *Evaluation Question #8*.

The EPA states that "[k]asugamycin is classified as practically non-toxic to freshwater and estuarine/marine fish and invertebrates on an acute exposure basis" (U.S. EPA 2013). Kasugamycin has low toxicity to fish as the LC50 for carp is 40 ppm over a period of 48 hours. The value for goldfish is the same (PubChem 2020a). For rainbow trout, acute toxicity over 96 hours was LC50 >120 ppm. For fathead minnow, the value was LC50 >110 ppm (NYS 2015).

Toxicity to the water flea, *Daphnia pulex*, is LC50 >40 ppm over a six-hour period (PubChem 2020a). For the water flea, *Daphnia magna*, EC50 for immobilization over 48 hours was >66.2 ppm (NYS 2015).

Kasugamycin has low toxicity to marine invertebrates and saltwater fish. For sheepshead minnow, *Cyprinodon variegatus*, the LC50 over a 96-hour exposure was >110 ppm. For mysid shrimp, *Americamysis bahia*, the LC50 over 96 hours was >100 ppm (NYS 2015).

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

Possible human health effects of kasugamycin cover both potential acute and chronic toxicity, reproductive problems, birth defects, and cancer, as well as potential antibiotic resistance in human pathogens.

Kasugamycin has low acute and chronic toxicity to mammals. Human doses are excreted quickly, mostly in the urine, and kasugamycin is not mutagenic and not a likely human carcinogen. More detail is given in *Evaluation Question #5*. Based on these findings, the EPA established a chronic dietary reference dose (RfD) for kasugamycin in humans of 0.113 mg/kg/day (U.S. EPA 2020).

Based on tolerances of 0.04 ppm for tomatoes and 0.2 ppm for pome fruit (Fed Reg 2014), the EPA estimated that likely kasugamycin exposure to the U.S. population as a whole was less than one percent of the RfD. The greatest exposure was in one- to two-year-olds, and this was less than 1.7 percent of the RfD (Fed Reg 2014; NYS 2015).

The Kasumin formulations include inerts and a preservative and have more toxicity warnings than the technical kasugamycin hydrochloride evaluated by the EPA in 2005. Kasumin 2L contains 2.3 percent kasugamycin hydrochloride hydrate (CAS 19408-46-9), 4.85 to 5 percent secondary alcohol ethoxylate (CAS 84133-50-6), and 0.1 percent 1,2-benzisothiazolone (CAS 2634-33-5) (Kasumin 2015). All of these are skin irritants. The thiazolone can cause serious eye damage, is a skin sensitizer, "may cause an allergic skin reaction," and can cause acute aquatic damage. The Safety Data Sheet warns that the formulation "may damage fertility or the unborn child" and is "very toxic to aquatic life" (Kasumin 2015). These inerts would not be allowed in an organic formulation. They do not appear on EPA List 4 (U.S. EPA 2004). The fertility and birth defect caution are for technical kasugamycin hydrochloride, discussed above and in *Evaluation Question #5*.

Occupational risks from application of Kasumin 2L are not of a concern if label directions for protective equipment are followed. These include long sleeves, long pants, chemically resistant shoes, socks, and gloves. Additional protection includes protective eyewear and a NIOSH approved respirator. The label requires a reentry interval of 12 hours. Exposure to residues from commercial applications to residential fruit trees is not a concern (Canada 2012).

Accidental poisonings—assumedly via ingestion—have apparently occurred because PubChem lists emergency detox procedures. Poisonings cause respiratory distress and pulmonary edema. Seizures may also occur, which are treated with diazepam. Hypovolemia is treated with Ringers solution. If kasugamycin solutions make contact with the eyes, they are to be treated with saline (PubChem 2020a).

From evaluation of all the toxicology tests, the EPA concluded 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" (Fed Reg 2014).

Antibiotic Resistance

Antibiotic resistance is a human health problem. According to the Centers for Disease Control (CDC 2013), at least two million people in the United States experience serious bacterial infections that are resistant to at least one type of antibiotic. At least 23,000 die as a direct result of these infections, while others die from conditions that were worsened due to infections with antibiotic-resistant bacteria.

Though kasugamycin is an antibiotic and has been used in human and veterinary medicine in the past, at present "there are no human or veterinary uses of kasugamycin as an antibiotic" (U.S. EPA 2013). Although classical toxicology tests suggest that human health effects from label applications of kasugamycin are not likely, there could be a possibility of antibiotic resistance or cross resistance. This problem has been seen with streptomycin. According to Sundin and Bender (1996), "[streptomycin-resistant] gene transfer events between human, animal, and plant associated bacteria have occurred."

Bacteria become resistant through antibiotic exposure in medicine and in agriculture. Major agricultural exposure comes from feeding antibiotics to animals to increase their growth. Antibiotics are also used to control plant disease in crops (CDC 2013). At least 40 percent of total antibiotic use is in animal feed, but antibiotics used on plants in the U.S. are less than 0.5 percent of the total (McManus et al. 2002).

Antibiotic Resistance from Crop Applications

Antibiotic resistance from antibiotics in animal feed is well established; the CDC would like the addition of antibiotics to animal feed to promote growth to be stopped (CDC 2013). Less research has been conducted on antibiotic-resistant pathogens produced from sprays for plant disease, but the NOP removed streptomycin and tetracycline from the National List, partly due to human health concerns (USDA 2014). Human bacteria could become resistant through exposure from accidents or spray drift, and through dietary exposure. Orchard workers would have the greatest risk of antibiotic resistance from sprays, while the greatest dietary exposure to kasugamycin is about 1.7 percent of the reference dose in one- to two-year-olds (NYS 2015; U.S. EPA 2013).

The Chronic Risk Quotient for mammals grazing in treated orchards is exceeded by kasugamycin sprays (see *Evaluation Question #8*). Resistant pathogens have been found in animals grazing on orchard grass treated with label rates of streptomycin (Scherer et al. 2013). Orchard antibiotic sprays could encounter pathogens in animal manure used as fertilizer. The Kasumin label does not allow animals to graze in treated areas or application of manure where contact with the antibiotic is possible (U.S. EPA 2020).

Antibiotic sprays could encounter human pathogens in the environment. "Opportunistic animal pathogens such as *Pseudomonas aeruginosa* and *Burkholderia* (*Pseudomonas*) *cepacia* are ubiquitous in the environment and strains of both species are known to be phytopathogenic" (Sundin and Bender 1996). Some clinical isolates of *Pseudomonas aeruginosa* are resistant to most antibiotics (Livermore 2002). Orchard sprays might cause resistance in epiphytic bacteria that then cause resistance in other bacteria through transmission of mobile genetic elements such as plasmids and transposons (von Winterdorf et al. 2016).

Spraying Orchards Can Cause Resistant Bacteria

Could kasugamycin orchard sprays lead to bacteria that harbor antibiotic resistance genes? Such is the case with other antibiotics. Spraying orchards with streptomycin and tetracycline can lead to resistant bacteria, such as *Pantoea agglomerans*. These bacteria can release transposons and plasmids that can confer resistance to environmental pathogens such as *Erwinia amylovora*. The same genetic elements that cause resistance in plant pathogens can cause resistance in human pathogens (McGhee et al. 2011; O'Brien 2002; Sundin 2002; Sundin and Bender 1996; USDA 2014).

Erwinia amylovora, the organism that causes fire blight, has developed field resistance from repeated applications of streptomycin to apple and pear orchards (Sundin 2014). Fire blight resistance to

streptomycin can come from resistance genes on transposon Tn5393 from *Pantotea* sp. But it can also come from mutations in the *Erwinia* chromosome and resistance genes strA and strB on plasmids. "The strA and strB genes and Tn5393 are widely distributed among gram-negative bacterial pathogens of humans, animals, and plants, and among environmental bacteria from many diverse habitats" (Forster 2015).

As might be expected from a complex phenomenon, research results are sometimes contradictory. Resistance genes to streptomycin have been found in treated orchards in Germany. But another study found no difference between treated and untreated German orchards. Studies have often not been replicated or are lacking in controls (Yashiro and McManus 2012). Yashiro and McManus (2012) even found a higher level of streptomycin resistance in bacterial populations of unsprayed orchards. Yashiro and McManus (2012) concluded that the unsprayed orchards had high levels of *Pseudomonas* and *Sphingomonas* that were already resistant to streptomycin.

Stockwell and Duffy (2012) cite several experiments where environmental sprays of antibiotics did not lead to resistance in environmental microbes. Some of the antibiotics used were inactivated by soil.

Animal Exposure to Orchard Sprays

About half of orchard antibiotic foliage sprays drift away and land on soil or nearby vegetation (NYS 2015; U.S. EPA 2005). Kasugamycin has not been evaluated to determine if its use for orchard sprays would lead to kasugamycin-resistant pathogens in animals grazing orchard grass, but spraying orchard grass with streptomycin at concentration levels used for fire blight leads to an increase in antibiotic-resistant human pathogens found in sheep grazing on sprayed grass. Before spraying, feces of control group sheep that subsequently grazed on untreated grass had 15.8 percent streptomycin resistant *E. coli*. The feces of the test group had 14.7 percent resistant *E. coli*. After spraying, levels were 22.3 percent in controls and 39.9 percent in the treated group. The streptomycin resistant *E. coli* was also resistant to several other antibiotics used to treat humans, including ampicillin and tetracycline. Streptomycin resistant *Staphylococcus* was found in nasal cavities of the treated sheep (Scherer et al. 2013). To prevent pathogens developing from kasugamycin sprays, the Kasumin label bans animal grazing in treated orchards (U.S. EPA 2018).

Scherer et al. (2013) speculate that streptomycin "may also have effects similar to those observed in sheep on people working in streptomycin treated orchards or living in their vicinity."

Kasugamycin Sprays

McGhee and Sundin (2011) found kasugamycin resistance in 401 bacterial isolates from apple flowers, leaves, and soil samples in orchards treated with Kasumin. Tancos et al. (2017) were not able to find kasugamycin resistant epiphytic organisms in New York apples, but the antibiotic changed the microbial spectrum in the orchard; see *Evaluation Question #8*.

Yoshii et al. (2012) reported that while "Spontaneous KSM [kasugamycin]-resistant mutants of *E. amylovora*, *E. coli*, and *Bacillus subtilis* harbored mutations in the ksgA methyltransferase gene," the possibility of field resistance due to these mutations is low because resistant mutants have low fitness due to decreased growth rate and virulence.

Resistance to kasugamycin in plant pathogens can be transferred from bacteria in the environment that have been exposed to the antibiotic. Aminoglycoside resistance often comes from aminoglycoside N-acetyltransferase mostly encoded by plasmids (Yoshii et al. 2012). Resistance of rice pathogens to kasugamycin came from a gene causing acetylation of kasugamycin. This gene aac(2')-IIa is located on the bacterial chromosome and was likely acquired by horizontal transfer. This gene is specific to kasugamycin and did not alter other aminoglycosides (Yoshii et al. 2012).

Kasugamycin has only weak activity against a number of human pathogens (Tamamura and Sato 1999). Although transfer of resistance genes between microbials is common, Stockwell (2014) states that "a direct link between antibiotic use in orchards and antibiotic resistance in human pathogens has not been demonstrated." However, the author does not cite any experiments where anyone evaluated this possibility. The Kasumin formulation has only been registered since 2018, and kasugamycin has been used

for only a short time in orchards. Further, "the complexity of bacterial population biology and genetics makes it practically impossible to trace bacteria (or resistance factors) from the farm to the hospital, or to directly attribute some fraction of new infections to agricultural antibiotic use" (Smith et al. 2005). However, an epidemiological study of antibiotic resistant microbes in organic orchard workers versus those in conventional orchards would be a good start.

Concern for antibiotic resistance led the NOSB Crops Subcommittee to ask the following question:
To what class of antibiotics does kasugamycin belong? Are there members of that class that are used in animal or human health and is there any evidence of cross reactivity of that class with other classes used in animal or human health?

Kasugamycin is an aminoglycoside antibiotic. Many members of this class including streptomycin, neomycin, kanamycin, gentamicin, and others are used as human clinical drugs or in veterinary medicine. Cross-resistance, which occurs when bacterial resistance to one antibiotic causes resistance to another, has been reported within the aminoglycoside class. For instance, kanamycin can be cross-resistant with other aminoglycosides (Rodriquez et al. 1999). Kanamycin can be cross-resistant with streptomycin (Chen et al. 2009). Gentamicin can be cross-resistant with other aminoglycosides (Gilleland et al. 1989; Houang and Greenwood 1977).

Cross-resistance has been found between aminoglycosides and other classes. For example, aminoglycosides and fluoroquinolones can be cross-resistant (Tsukamamoto et al. 2013). Tetracycline or ampicillin can be cross-resistant with kanamycin (Chen et al. 2009). Cross-resistance has been seen between aminoglycosides and beta-lactams (Sanders et al. 1984) and kasugamycin with blasticidin S (aminoacylnucleoside class) (Shiver et al. 2016). Fire blight isolates highly resistant to streptomycin also have reduced sensitivity to oxytetracycline (Adaskaveg et al. 2009).

Laboratory exposure of *Bacillus subtilis* to kasugamycin generated two kinds of resistant mutants. One was resistant to kasugamycin effects on protein synthesis. Another had no resistance to effects on protein synthesis but had weak cross-resistance with gentamycin and kanamycin (Tominaga and Kobayashi 1978).

Resistance can arise from changes in membrane permeability, prevention of drug binding, and enzymatic inactivation of the drug molecule. Genes for inactivation enzymes are often carried on bacterial plasmids or transposons. Plasmids and transposons are exchanged by related and unrelated bacteria with horizontal gene transfer (O'Brien 2002; von Wintersdorff et al. 2016).

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

Biological Controls
Biological controls (biocontrols) combined with sanitation, bloom reduction, and applications of copper can give satisfactory control of fire blight (Adaskaveg 2017a, 2017b, 2017c; Dupont 2019; Johnson and Temple 2013). Biocontrols include antagonists such as *Pseudomonas fluorescens* A506 isolated from pear (BlightBan® A506), *Pantoea agglomerans* C9-1 isolated from apple (BlightBan C9-1), *P. agglomerans* E325 isolated from apple (Bloomtime Biological™), *Bacillus subtilis* QST713 (Serenade®), and *Bacillus amyloliquefaciens* D747 (Double Nickel™). The best biocontrol by far for preventing fire blight is the yeast *Aureobasidium pullulans* (mixtures of strains DSM 14940 and DSM 14941), or Blossom Protect. Blossom Protect gives reliable results and is highly effective at preventing fire blight (Adaskaveg et al. 2017a; Sundin et al. 2009).

Biological control can be just as effective as sprays of kasugamycin. On Bartlett pears in California, there was no statistical difference in effectiveness between kasugamycin only, Blossom Protect, the copper treatment Cueva (copper octanoate), and Cueva plus the biological Serenade (*Bacillus subtilis*) (Adaskaveg et al. 2019a).

For Granny Smith apples under high disease pressure in California, Adaskaveg et al. (2019a) found that Blossom Protect plus acidic buffer was the most effective treatment. Disease incidence was reduced from 42.4 percent to 11.1 percent. Kasumin (kasugamycin) alone was slightly less effective than the biological.

Blossom Protect plus buffer was just as effective as streptomycin or oxytetracycline in Washington State University trials in 2013, 2014, 2016, and 2017. Kasugamycin had effectiveness similar to streptomycin and oxytetracycline in WSU trials 2006, 2009, 2010, 2011 (Dupont 2019).

Timing of sprays is an important part of effectiveness. Blight forecasting can help decide when to apply biological controls. At least one application should be applied early in the bloom cycle. Applications should also occur when blight forecasting predicts high likelihood of establishment. Treatments should be applied early in the disease risk period (Johnson et al. 2004). Applications should be at least twice between 25 and 90 percent bloom (Johnson and Stockwell 1998).

Some More Effective than Others

Some biocontrols are more effective than others. In Michigan, New York, and Virginia, Bloomtime Biological (28.5%) and Blight Ban C9-1 (33.1%) gave better control than Blight Ban A506 (12.5%). But there was a considerable range of effectiveness location to location and year to year (Sundin et al. 2009).

Adaskaveg et al. (2016) found that Blossom Protect with molasses was effective at high disease pressures for fire blight in California apples. Blossom Protect and copper treatments were more reliable as fire blight treatments in California apples than Double Nickel, Serenade, Regalia (extract of giant knotweed), Actinovate (*Streptomyces lydicus*), and Bloomtime Biological (Adaskaveg (2017b).

Antagonists—those biological control agents acting directly on target pests—were more effective at reducing infections than application of avirulent strains of *Erwinia* (Johnson et al. 2009). Freeze dried applications of antagonists were more effective than application of freshly prepared inoculant (Stockwell et al. 1998).

Biocontrol More Effective in the West

Fire blight biocontrols work better in the western U.S. than in the East. In Michigan, New York, and Virginia, average blight reduction using Blight Ban A506 was about 12 percent. In California and Oregon, A506 fire blight reduction was 40–60 percent. Average fire blight reduction using Blight Ban C9-1 in the East was about 26 percent. In the West standalone treatments were about 40–60 percent effective. The difference is thought to be due to better flower colonization in the West (Sundin et al. 2009). Because more than 90 percent of organic apples are grown in the West, and more than 71 percent are grown in Washington, biocontrol is a major factor in organic apple production (Granatstein and Kirby 2019).

For instance, in Oregon pears, application of *P. agglomerans* C9-1 (Blight Ban C9-1) at 70 percent bloom reduced fire blight 51 percent in orchards deliberately inoculated with *Erwinia*. In Oregon apples, Blight Ban C9-1 reduced incidence of fire blight by about 49 percent (Johnson et al. 2008). Average fire blight reduction of Blight Ban C9-1 in the East is about 26 percent (Sundin et al. 2009).

Temperature and Mixtures

Temperature is an important part of colonization competence. Blight Ban A506 is generally less effective than Blossom Protect or Double Nickel. The optimum temperature for *Erwinia* is 24–29°C. BlightBan A506 works better at lower temperatures, 15–20°C. Optimum temperature for Double Nickel is 20–35°C, for Blossom Protect 15–30°C. Blossom Protect and Double Nickel are optimally effective over a larger range of temperatures that coincide with the temperature profile of *Erwinia* (Adaskaveg et al. 2017a; Sundin et al. 2009).

Mixtures of the two BlightBans (A506 and C9-1) controlled fire blight on pears in Washington and Oregon. The antagonists reduced growth and establishment of *Erwinia* on blossoms. Sprays were applied either two- or four-times during bloom. Effectiveness was greatest with established concentrations of antagonists

>10^5 cfu/blossom (Johnson et al. 1993). When using mixtures, care must be taken to choose compatible microbes. Some biocontrols are mechanistically incompatible (Stockwell et al. 2011).

Integrated Organic Programs

The major mechanism of fire blight biological control is competitive exclusion. Stockwell et al. (2008) pioneered an integrated pest management (IPM) approach where a biocontrol was first used in early bloom time to colonize flowers in competition with *Erwinia*. Then an antibiotic was applied at full bloom or petal fall. The biological control, *P. agglomerans* (Bloomtime), was used first followed by oxytetracycline. This suppressed fire blight, but oxytetracycline cannot be used in organic agriculture. An all-organic method was Bloomtime followed by *Bacillus subtilis* (Serenade). This combination was also effective, but twice as many Serenade sprays were needed compared to oxytetracycline, driving up the cost (Johnson and Temple 2013).

Adaskaveg et al. (2017a) tried many different biological controls in California apple experiments. Blossom Protect or *Bacillus amyloliquifaciens* (Double Nickel) effectively reduced fire blight. *Bacillus subtilis* (Serenade Opti) plus the copper product Badge X2 gave a significant fire blight reduction (Adaskaveg 2017a). New materials mentioned that show promise include bacteriophages (Adaskaveg et al. 2019a).

Antagonists must be compatible with chemicals used for fire blight suppression. Streptomycin suppressed "populations of indigenous bacterial epiphytes" but had little effect on establishment and spread of the biocontrol Blight Ban C9-1 (Johnson et al. 2000). Copper (see below) is compatible with yeast but not with bacterial antagonists (Adaskaveg 2019b).

Biocontrol microbials are generally thought to produce few problems with health. Many of them are ubiquitous in the environment. Some microbials can cause clinical infections in those with compromised immune systems. Label directions should be followed when they are applied (Mittal et al. 2018; Quarles 2013).

Copper Treatments

Forms of copper used in apple production include copper sulfate, fixed copper, and soluble copper. Copper sulfate is so soluble it must be mixed with lime to prevent phytotoxicity. Fixed coppers, including copper hydroxide, copper oxychloride and others, are nearly insoluble in water. Applied as dormant treatments, they slowly release copper ion, preventing *Erwinia* from colonizing orchards from overwintering cankers. Soluble coppers such as the copper soap, Cueva, are sometimes applied during the blooming season because copper content of the formulations is low and less likely to cause phytotoxicity (Dupont 2019).

Copper is less effective at high disease pressures because it does not kill the pathogen, only inhibits its growth. Only low concentrations of copper are registered for fire blight, and the *Erwinia* organism showed moderate resistance in California (Adaskaveg et al. 2019a).

Compatibility is a consideration. "Copper is generally incompatible with bacterial biocontrols, but compatible with yeast based products" (Adaskaveg et al. 2019b). However, products such as Cueva with low copper ion concentration are compatible with *Bacillus* based biocontrols in tank mixes (Dupont 2019).

Fixed coppers such as copper hydroxide and copper oxychloride have a long residual time and copper ions are released slowly from the insoluble material. These are often applied during the dormant period. Because of phytotoxicity, fixed coppers should not be applied with the induced material Fosphite® (see below) or with the Buffer Protect used to enhance the Blossom Protect™ biological control (Dupont 2019).

Soluble coppers such as copper octanoate (Cueva) or Previsto (copper ions in an alginate matrix) are formulated at much lower copper concentrations than the fixed copper materials. Because trees are exposed to less copper ion, these formulations can be applied outside the dormant period. Cueva is compatible with *Bacillus* based biocontrols in tank mixes. Cueva and Previsto gave about 60–70 percent fire blight control in Washington State University trials (Dupont 2019).

Elkins et al. (2015) applied copper products in the "green tip" physiological stage, which occurs about five weeks before full bloom. Application at this time prevents pathogen spread from overwintering cankers. In California pears, horticultural oil plus copper was compared to horticultural oil only. Copper plus oil produced a sanitation effect, slowing movement of *Erwinia* from trunk cankers to blooms. Fruit quality was not affected by the copper treatment.

Copper is most often used as a dormant spray, and copper persists on surfaces. However, heavy rainfall (three inches or greater) washes off all the copper. Application of copper to cankers with the surfactants Pentrabark or Regulaid was ineffective in reducing bacterial populations (Acimovic and Meredith 2017a).

Shoot Blight Control

Low rates of copper can be used for shoot blight control during the summer. But Acimovic et al. (2017a) found that two sprays of 0.196 lb copper per acre provided poor fire blight protection when applied as bloom time sprays in New York apples. Once bacteria enter and "establish in flowers, shoots, and wood tissue, sprayed bactericides have no effect."

Fire blight infection of blooms is less common in Illinois due to the cooler climate and shorter bloom periods. Instead, shoot blight infections are more common due to trees being damaged during storms. In Illinois apple orchards, Kocide (copper hydroxide) combined with either mancozeb or oxytetracycline was not effective in reducing shoot blight infection. Other materials such as streptomycin, *P. fluorescens* A506, and *Bacillus subtilis* QST713 (Serenade) were also not effective, but ksugamycin controlled shoot blight (Jurgens and Babadoost 2013).

Induced Resistance

Some success has been seen with sprays, trunk paints and injections of materials that cause systemic acquired resistance (SAR). Plants have immune systems controlled by salicylic acid. Salicylic acid causes release of proteins that fight infection. SAR materials that have been tested for fire blight include acibenzolar-S-methyl (ASM), Regalia (extract of giant knotweed, *Reynoutria sachalinensis*), salts of phosphorous acid (Fosphite), and others (Acimovic 2015; Johnson and Temple 2016). Regalia is allowed in organic agriculture; the active ingredients for ASM and Fosphite would have to be added to the National List of Allowed Synthetics.

Foliar sprays or injections of acibenzolar-S-methyl can reduce fire blight infections. Optimal use of ASM may be to prevent the spread of fire blight from areas of excised cankers. Johnson and Temple (2016) tested root drenches, trunk paints or foliar sprays on greenhouse apple trees grown in pots. Trees were inoculated with fire blight. ASM treatments reduced canker expansion by 22–25 percent. Root drenches were more effective before inoculation, trunk paints were most effective at inoculation, foliage sprays had variable timing effects. Trunk paints were the most effective application method. Painting pruning areas could help reduce infection as trees are pruned to remove infection (Johnson and Temple 2016).

In field experiments with 3- to 14-year old apple and pear trees, painting the pruning site with ASM "yielded 62% less diseased wood" over a five-year period (Johnson and Temple 2017).

Air blast sprayer losses of liquid formulations into the environment are 44–71 percent (Acimovic 2015). To reduce losses of SAR materials, trunk injections can be used instead of sprays. Trunk injections of SAR materials into the xylem in some circumstances can reduce shoot blight. Injections cause production of chitinase and glucanase enzymes that destroy *Erwinia*. In New York apples, trunk-injected Fosphite produced 55.9 percent fire blight control, similar to streptomycin (61 percent) when disease pressure was moderate. ASM produced statistically similar results (42.2 percent). Control with Fosphite was less (25.1 percent) under high disease pressure. Resistance genes were expressed less in flowers than leaves. Fire blight suppression was better on shoots than flowers.

Acimovic and Meredith (2017b) tried foliar sprays and trunk injections of induced resistance materials on apple trees in New York under high disease pressure. Materials included Regalia (*Reynoutria sachalinensis*),

Prestop (*Gliocladium catenulatum* J1446), *B. amyloliquefaciens* F727, mixtures of copper (CS2005) and Regalia, streptomycin and oxytetracycline. Overall, the induced materials provided poor blossom and shoot blight control. Regalia and *B. amyloliquefaciens* produced the most russeting.

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

Fire blight spreads in orchards from overwintering cankers on the trees. The bacterial pathogen, *Erwinia amylovora*, is dispersed by insects and rainfall to blossoms. Infected blossoms and trees wounded by storms in the spring produce endophytic populations of bacteria, causing infected fruit and summer outbreaks of rootstock and shoot blight. New cankers form on branches and stems in autumn, completing the life cycle (Dupont 2019; Norelli et al. 2003).

The alternative to kasugamycin is an integrated organic program that attacks fire blight at every point in its life cycle. As part of the program, resistant species of apples and pears are planted. Unfortunately, most of the commercial varieties popular with consumers are susceptible to fire blight. Growers are not inclined to plant resistant species unless produce is commercially viable. However, there has been a movement toward using resistant rootstocks. Resistant rootstocks can help prevent rootstock blight that occurs at the graft union (Norelli et al. 2003).

Other components of an integrated organic program are cultural controls that include pruning, no irrigation during bloom time, and proper management of weeds and cover crops to reduce relative humidity (Dupont 2019; Pfeiffer 2017). Effectiveness of pruning can be increased by application of induced systemics (Johnson et al. 2017; Dupont 2019). See *Evaluation Question #11* for more information.

Cultural controls can be combined with application of fixed copper sprays in dormant and prebloom periods, thinning of blossoms, application of biological controls such as Blossom Protect during bloom time, and application of biocontrol antagonists such as Serenade later in the blooming period (Adaskaveg 2017a, 2017b, 2017c; Johnson and Temple 2013). The integrated organic program can reduce the incidence of fire blight in apples by 90 percent or more (Johnson and Temple 2013).

Much better results are obtained by the addition of biocontrols to an integrated organic program. In Oregon, organic management of apples and pears gave results equal to conventional management. The best treatment in apples was two applications of lime sulfur at 30 percent and 70 percent bloom followed by two sprays of *Aureobasidium pullulans* (Blossom Protect). Lime sulfur alone reduced fire blight by 48 percent. Lime sulfur plus Blossom Protect gave a 91 percent reduction. This all-organic program was just as effective as a single spray of streptomycin (Johnson and Temple 2013).

Lime sulfur cannot be used in pears because of russeting. But in Bartlett pears, Golden Delicious, Gala, and Rome Beauty apples, two applications of Bloomtime Biological in early bloom followed by Blossom Protect at full bloom gave 86 percent fire blight reduction (Johnson and Temple 2013).

Copper sprays, by sanitizing surfaces and inhibiting reproduction of *Erwinia*, help prevent the spread of fire blight from cankers to blooms. The yeast Blossom Protect colonizes blossoms and prevents infection of *Erwinia* mainly by competitive exclusion. The antagonist *Bacillus subtilis* QST713 (Serenade) produces antibiotic substances that suppress the pathogen later in the blooming period (Johnson and Temple 2013; Stockwell et al. 2008); see *Evaluation Question #11*.

Thinning blossoms alone can reduce incidence of fire blight in apples by about 50 percent (Johnson and Temple 2013). Thinning can be done by hand, but it is very labor intensive. Lime sulfur was used successfully for blossom thinning in Oregon apples, and researchers recommend that registration of lime sulfur for thinning should be pursued in all states where organic apples are grown (Johnson and Temple 2013).

The integrated organic program works best on the West Coast where biocontrols are more effective. It works better for apples than pears because due to russeting, lime sulfur cannot be used on pears to thin blossoms (Johnson and Temple 2013). See *Evaluation Question #11* for more information.

Focus Areas Requested by NOSB

1. ***To what class of antibiotics does kasugamycin belong? Are there members of that class that are used in animal or human health and is there any evidence of cross reactivity of that class with other classes used for animal or human health?***

As mentioned in *Evaluation Question #10,* kasugamycin is an antibiotic in the aminoglycoside class. Other members of this class include streptomycin, neomycin, and kanamycin—antibiotics that are often used in medicine and in veterinary practice (CDC 2013).

Cross resistance occurs when bacterial resistance to one antibiotic causes resistance to another. Cross-resistance between members of the aminoglycoside class has been documented. Cross-resistance between kasugamycin and other aminoglycosides has not been seen (Chen et al, 2009; Gilleland et al. 1989; Rodriguez et al. 1999).

Cross-resistance between aminoglycosides and antibiotics of other classes has been found (Chen et al. 2009; Sanders et al. 1984; Tsukamamoto et al. 2013). Cross-resistance between kasugamycin and members of other antibiotic classes is extremely rare. But cross-resistance has been seen between kasugamycin and blasticidin S, an aminoacyl nucleoside antibiotic (Shiver et al. 2016).

2. How does the timing (i.e. bloom, petal fall, post bloom) of kasugamycin application affect the potential for residue in the fruit at harvest and are there any residues of this antibiotic in fruit at harvest?

Kasugamycin is applied only while the fruit trees are in bloom. There is a 90–day preharvest interval for pome fruit that is mandated by the Kasumin pesticide label (U.S. EPA 2018). During this time, residue levels drop to about 1/1000 of the amounts applied (PubChem 2020a; NYS 2015). Tolerance levels for kasugamycin on apples, pears, and other pome fruit are 0.2 ppm (Fed Reg 2014). Residues on fruit at harvest should range between 0.02 and 0.2 ppm (Stockwell 2014). These concentrations are well below established EPA toxicity thresholds. Whether these very low residue levels can contribute to antibiotic resistance in human pathogens is unknown or uncertain. One mitigating factor is that kasugamycin is not used in humans or in veterinary medicine (U.S. EPA 2013).

More detailed answers to this question are given in *Evaluation Question #4*.

3. Is this product susceptible to development of resistance with normal (labelled) use?

Kasugamycin has been in agricultural use since 1965. It has been used against a number of plant pathogens. In every instance, some level of resistance has occurred (Vallad et al. 2010; Yoshii et al. 2012). The EPA believes that resistance of the fire blight pathogen *Erwinia amylovora* to kasugamycin is possible, and the Kasumin label requires a resistance management plan. This plan includes use of kasugamycin as part of an IPM program and less than four applications per year (U.S. EPA 2018).

Resistance of epiphytic bacteria and soil bacteria in orchards has been seen. There is some concern that these resistant bacteria might act as a harbor for mobile genetic elements such as transposons that could cause the pathogen *Erwinia amylovora* to become resistant (McGhee and Sundin 2011).

More detailed answers to this Question are given in *Evaluation Question #8*.

4. ***Compare the positive and negative impacts of alternatives with the potential positive and negative impacts of the use of kasugamycin.***

Positive and Negative Impacts of Alternatives

Positive impacts of using an integrated organic program are that it can be very effective, and components of the program have low negative effects on the environment. The current all-organic program can be more than 90 percent effective in preventing fire blight in apples on the West Coast where 90 percent of organic apples are grown (Granatstein and Kirby 2013; Johnson and Temple 2013).

There are negative impacts of using an integrated organic program. Lime sulfur, a synthetic active approved for use in organic production for plant disease control, cannot be used on pears. Growers must use hand thinning, which is labor intensive. Antagonists such as *B. subtilis* (Serenade) are used in integrated organic programs. Repeated sprays of *B. subtilis* toward the end of bloom can drive up costs (Stockwell et al. 2008; Johnson and Temple 2013).

In eastern states, biocontrol is less effective. Growers are experimenting with sprays of soluble copper during the summer, and applications of induced systemic materials. Results have been less reliable than situations where biocontrols have good efficacy. Fire blight can still be managed except in cases of high disease pressure (Acimovic 2015; Acimovic 2017a; Acimovic 2017b). Researchers in California have identified moderate copper resistance in the fire blight bacteria, which lessens the effectiveness of copper (Adaskaveg 2019a). Copper is an elemental metal, which is persistent in the environment. Intensive use can result is elevated soil levels in some soil types and regions (USDA 2011). For more information, see *Evaluation Question #11.*

Organic growers have been forced to develop alternatives to antibiotics only since 2014. There is a learning curve, and new materials such as phages have been introduced (Adaskaveg 2019a).

Positive Impacts of Kasugamycin

Kasugamycin is relatively inexpensive and effective because the fire blight organism has not developed resistance. In eastern states where biocontrols are less effective, and in pear production, where some components of integrated production are not possible, it could be extremely useful (California Apple Commission 2020).

If kasugamycin is approved, growers would apply it instead of the second biocontrol in the integrated organic program. Kasugamycin would be a less expensive alternative. Limiting use of kasugamycin to sprays late in bloom as part of an integrated program would delay the onset of fire blight resistance (Johnson and Temple 2013; Stockwell et al. 2008). For more information, see *Evaluation Question #8.*

Negative Impacts of Kasugamycin

Fire blight has grown resistant to every antibiotic used against it. There is good reason to believe fire blight will become resistant to kasugamycin. The NOSB and NOP identified several reasons to stop the use of streptomycin in organic production: resistance is widespread; some organic markets do not allow the use of antibiotics on apples and pears; and "organic integrity and sales are threatened because of consumer expectation that antibiotics are not used in organic production" (USDA 2014). These arguments summarize some of the potential negative impacts of kasugamycin. For more information, see *Evaluation Question #8.*

Kasugamycin is also phytotoxic. Plant damage limited early use of kasugamycin for fire blight (Adaskaveg et al. 2011). The Kasumin formulation is less phytotoxic than other formulations and is more effective (McGhee and Sundin 2011). But there is no guarantee that an organic formulation will be as effective as Kasumin.

Though effective residues of kasugamycin quickly decay, it is classified as moderately persistent to persistent in the environment (U.S. EPA 2013). According to the EPA (2013), effects on environmental microbes are unknown and uncertain. Kasugamycin sprays, however, are known to change the microbial spectrum of orchards. For more information, see *Evaluation Question #4.* Environmental effects are generally low level. But the EPA Chronic Risk Quotient for mammals grazing in treated orchards would be exceeded. Also, some aquatic plants could be threatened. For more information, see *Evaluation Question #9.*

Tancos et al. (2017) found the increased numbers of *P. agglomerans* a concern, as this microbe is known to transfer a transposon that carries antibiotic resistance. For more information, see *Evaluation Question #8.*

There is evidence that antibiotic use in animal feed can lead to antibiotic resistant human pathogens. The likelihood of kasugamycin use causing antibiotic resistance in human pathogens is uncertain. According to Stockwell (2014), "a direct link between antibiotic use in orchards and antibiotic resistance in human pathogens has not been demonstrated." However, a direct link has been found with sheep grazing on orchard grass treated with streptomycin. Sheep in treated orchards had greater numbers of antibiotic resistant *E. coli* and *Staphylococcus* than those grazing on untreated grass (Scherer et al. 2013). According to the EPA (2013), the likelihood of a plant antibiotic causing resistance in a human pathogen is low, but not zero. Designing experiments to test this possibility would be very difficult (Smith et al. 2005). With this said, an epidemiological study of antibiotic resistant microbes in organic orchard workers versus those in conventional orchards would be a good start. For more information, see *Evaluation Question #10.*

Report Authorship

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