Streptomycin
Crops

Identification of Petitioned Substance

Chemical Names:
O-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1->2) -*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1->4)-N,N′-bis(aminoiminomethyl)-D-streptamine

Other Names:
Streptomycin A
Streptomycine
Streptomycinum
Streptomycin sulfate
Streptomycin sesquisulfate

Trade Names:
Ag streptomycin
Agri-mycin 17
As-50
Bac-master
Ferti-lome fire blight spray
Firewall 17WP
Rg s 50 wp
Streptomycin 17

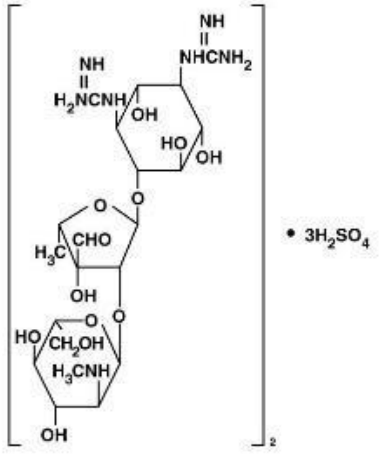
CAS Numbers:
57-92-1
3810-74-0 (streptomycin sulfate)

Other Codes:
006306 (US EPA PC code)
006310 (US EPA PC code - streptomycin sulfate)

Characterization of Petitioned Substance

Composition of the Substance:
Streptomycin ($C_{21}H_{39}N_7O_{12}$) is a bactericidal, aminoglycoside antibiotic derived from the soil bacterium *Streptomyces griseus*. It is used in human and veterinary medicine to treat bacterial infections and in agriculture to control bacterial diseases of many different crops and ornamental plants. It is marketed as the sulfate salt of streptomycin [$2(C_{21}H_{39}N_7O_{12})\bullet 3(H_2SO_4)$]. The molecular structure of streptomycin sulfate is shown in Figure 1.

Figure 1. Molecular Structure of Streptomycin Sulfate



Streptomycin sulfate is an ionic compound that dissociates into positively charged streptomycin and negatively charged sulfate ions in aqueous solution. In this document, "streptomycin" refers to both streptomycin and streptomycin sulfate. The Pesticides Action Network (PAN, 2010) lists currently registered pesticide products for streptomycin sulfate, but none for streptomycin. The Organic Materials Review Institute (OMRI, 2011) lists only one streptomycin product, Agri-Mycin® 17, which contains 22.4% streptomycin sulfate (equivalent to 17% streptomycin).

Properties of the Substance:
Streptomycin sulfate generally exists in the form of a white to tan powder that is easily soluble in water (EPA, 2006b). It is odorless or nearly odorless with a slightly bitter taste (HSDB, 2002). Agricultural streptomycin is most commonly produced as a wettable powder, dust, and soluble concentrates. Medicinal streptomycin is most commonly produced as a liquid for injection.

The salt forms of streptomycin absorb moisture from the air, but are stable in air and on exposure to light. Streptomycin is a polar compound, highly soluble in water, and unstable to heat (HSDB, 2002). Neutral solutions of streptomycin kept at temperatures below 25ºC are stable for weeks. Streptomycin is more active at an alkaline pH, and it is unstable in strong acids and bases. (EXTOXNET, 1995).

Specific Uses of the Substance:
Streptomycin is currently included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) as a synthetic substance allowed in organic crop production for fire blight control in apples and pears only [7 CFR 205.601(i)(11)]. Fire blight is a destructive bacterial disease that affects certain species in the Rosaceae family (Koski and Jacobi, 2009). It is caused by the bacterium *Erwinia amylovora*, which is capable of infecting blossoms, fruits, vegetative shoots, woody tissues, and rootstock crowns (Norelli et al., 2003). Streptomycin is one of many control agents currently used to prevent the spread of fire blight on organic and conventional apple and pear orchards. It is typically applied by ground spray in the spring according to weather and crop development. Spraying begins at early bloom and may be repeated every 3 to 4 days (EPA, 2006b). The timing of application is critically important to prevent infection. Once the disease spreads from the blossoms, there are no available cures. Streptomycin and other chemical sprays have little effect after the onset of symptoms (Koski and Jacobi, 2009). According to Sundin et al. (2009), streptomycin is still the most effective agent available to growers for limiting blossom populations of *Erwinia amylovora*. However, streptomycin-resistant strains of the pathogen are now present in many regions of the U.S. decreasing the efficacy of this agent.

In addition to controlling fire blight in apples and pears, streptomycin is used to control bacterial diseases of many other fruits, vegetables, seeds, and ornamental crops. While the majority of agricultural streptomycin is used on apples and pears, other crops include celery, philodendron, tomato, peppers, dieffenbachia cuttings, chrysanthemums, roses, pyracantha, potatoes, and tobacco (EPA, 2006b). Streptomycin is also registered with the U.S. Food and Drug Administration (FDA) to treat bacterial diseases in animals and humans. However, acquired resistance to streptomycin in human and veterinary pathogens is widespread, which limits its usefulness (Arias and Murray, 2009; Dowling, 2006). Livestock uses include treatment of enteric infections in poultry, swine, and calves. In human medicine, intramuscular injections of streptomycin are sometimes used alone or in combination with other antibiotics to treat tuberculosis, tularemia, plague (*Pasteurella pestis*), bacterial endocarditis, brucellosis, and other infections caused by gram-negative bacteria such as *Escherichia coli* and klebsiella species (NLM, 2006).

Approved Legal Uses of the Substance:
Streptomycin is a registered pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which is administered by the U.S. Environmental Protection Agency (EPA). It was first registered in 1955 for use in controlling bacterial and fungal diseases of certain agricultural and non-agricultural crops. EPA issued a Registration Standard for streptomycin in September 1988 (EPA, 1988), a Reregistration Eligibility Decision (RED) in September 1992 (EPA, 1992), and a Tolerance Reassessment Progress and Risk Management Decision (TRED) in June 2006 (EPA, 2006b). Streptomycin is currently under registration review by EPA which is scheduled to be complete in 2014 (EPA, 2009). A tolerance of 0.25 ppm has been established for residues of streptomycin in raw apple, pear, celery, pepper, tomato, and potato, while a tolerance of 0.5 ppm has been established for dry and succulent beans (40 CFR 180.245).

Streptomycin is regulated by FDA as a prescription drug. It is approved for use as an injectable solution. Veterinary use of streptomycin is also regulated by FDA. It is approved for use in veterinary medicine as an oral or injectable solution to treat bacterial enteritis caused by *Escherichia coli* and salmonella species as well as infections caused by leptospirosis species (21 CFR 520.154b, 21 CFR 520.2158a, 21 CFR 522.650). A tolerance of 2.0 ppm in kidney and 0.5 ppm in other tissues has been established for residues of streptomycin in uncooked, edible tissues of chickens, swine, and calves (21 CFR 556.610).

Action of the Substance:
Aminoglycoside antibiotics, including streptomycin, bind to bacterial cell components (ribosomes) and reduce their ability to correctly synthesize proteins needed for growth and survival. The result is accumulation of erroneous proteins and cell death (Hermann, 2007). In general, aminoglycosides and streptomycin are effective on many aerobic and gram-negative bacteria and some gram-positive bacteria. They are not useful for anaerobic or intracellular bacteria. Bacterial resistance to streptomycin can develop by three general mechanisms: decrease of intracellular streptomycin concentration (by blocking cellular entry or actively pumping it out of the cell), enzymatic modification of streptomycin making it less harmful to the cell, or, rarely, modification of streptomycin's target site preventing it from binding (Hermann, 2007).

Streptomycin can be phytotoxic to plants, therefore it is sprayed on the surface of plants rather than injected (McManus and Stockwell, 2000). Most apple and pear producers are prudent in their use of streptomycin sprays to reduce costs and to prevent the development of streptomycin-resistant strains of *Erwinia amylovora*. Disease-risk models help producers optimize the timing of antibiotic sprays and reduce the total number of applications. These measures can help reduce the development of antibiotic resistance.

Combinations of the Substance:
Agricultural streptomycin is not a precursor or component of any other substances on the National List. Tetracycline (oxytetracycline) is another antibiotic on the National List approved for use in control of fire blight. Apple and pear producers may alternate the use of these two antibiotics in different seasons. Also, there is evidence to suggest that some producers are applying these two antibiotics in combination to apple and pear trees when streptomycin-resistant strains are present in the orchard (Johnson, 2010). Copper sulfate, fixed copper mixtures (such as Bordeaux mix), and peracetic acid are all listed on the National List and may be used for control of fire blight in apples and pears. Based on recommendations, it is unlikely that producers are applying these in combination or close succession with streptomycin (Univ. of Illinois Dept. of Crop Sciences, 2005; Koski and Jacobi, 2009). Some biological control agents that are streptomycin resistant may be applied to organic apple and pear trees in combination or close succession with streptomycin (see response to Evaluation Question #11 for a description of the available biological control agents).

Status

Historic Use:
Streptomycin was first registered as a pesticide in the United States in 1955. Since that time, it has been used in conventional agriculture for control of fire blight in apples and pears along with many other bacterial diseases affecting fruits, vegetables, seeds and ornamental crops. It has been used in the U.S. in organic agriculture for control of fire blight in apples and pears for the past decade. The most recent renewal of streptomycin for use in organic agriculture was completed by the National Organic Standards Board (NOSB) in 2006.

OFPA, USDA Final Rule:
Streptomycin is included on the National List as a synthetic substance allowed in organic crop production for fire blight control in apples and pears only [7 CFR 205.601(i)(11)].

International
Streptomycin is not specifically listed for use by the Canadian General Standards Board, CODEX Alimentarius Commission, European Economic Community (EEC) Council Regulation, EC No. 834/2007

and 889/2008, International Federation of Organic Agriculture Movements (IFOAM), or the Japan Agricultural Standard (JAS) for Organic Production for control of fire blight or any other uses.

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

Evaluation Question #1: What category in OFPA does this substance fall 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?

A). Streptomycin is considered a toxin derived from bacteria.

B). The substance is a synthetic ingredient and is not classified by EPA as an inert of toxicological concern.

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)).

Streptomycin is a naturally occurring compound which is produced by the soil bacterium *Streptomyces griseus.* Agricultural streptomycin is produced on a large scale by aerobic fermentation of *Streptomyces griseus* followed by isolation and purification by ion exchange (HSDB, 2002; EPA, 1992). Agricultural antibiotics, including streptomycin, are formulated with water-insoluble carriers (e.g. kaolin clays) that adsorb the active ingredient (Rezzonico et al., 2009). No further information on the manufacture of agricultural streptomycin was identified.

The Indian Department of Scientific and Industrial Research (DSIR, 1991) reported the basic manufacturing process for medicinal streptomycin. Since there is no evidence to suggest a fundamental difference in the manufacture of agricultural vs. medicinal streptomycin, this information is provided in this technical report. The manufacturing process comprises three major steps: (1) preparation of inoculum (i.e., substance containing the microorganism), (2) fermentation, and (3) extraction, recovery, and purification. The first step is the preparation of inoculum from the original culture of *Streptomyces* species. The inoculum is transferred to a series of incubators where the total quantity of biomass is greatly increased and then to fermentation tanks. The growth medium contains suitable ingredients including a source of carbohydrates (e.g., glucose), a nitrogen source (e.g., soybean flour), and various salt solutions to provide nutrients to optimize growth and yield of streptomycin. The fermentation process usually takes about 200 hours. To extract the compound, the mixture is filtered to remove the bacteria, diluted, and passed through ion exchange resin columns where streptomycin is adsorbed. It is further treated with several chemicals (e.g. solvents, antifoaming agents), activated carbon, and de-ashed in the resin column to remove impurities. Streptomycin is typically extracted from the resin column as streptomycin sulfate. Purified streptomycin (or streptomycin sulfate) solution is then concentrated and dried.

Evaluation Question #3: Is the substance synthetic? 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).

Streptomycin is produced through a naturally occurring process (aerobic fermentation), but the processes used to isolate and purify the substance are not naturally occurring. Therefore, agricultural streptomycin is considered synthetic. See the response to Evaluation Question #2 for more details on the manufacturing process.

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)).

A certain background level of streptomycin is expected in soil due to the natural presence of the bacterium *Streptomyces griseus* (Brosché, 2010). EPA (1988, 1992) cited data that show that streptomycin biodegrades relatively quickly in soil and water. The breakdown products included methylamine, carbon dioxide, and urea, all of which occur naturally in the environment.

As no other environmental fate and transport data were submitted to the EPA, the Health Effects Division (HED) Chapter of the 2006 TRED reported that EPA employed an environmental fate estimation program (EPI Suite) to provide data for risk assessment. The results of the estimates as reported in EPA (2006a) are presented below:

> *Streptomycin has a very low Henry's law constant and is very highly soluble in water. The chemical is moderately persistent in aerobic soil (a single value of t1/2= 17.5 days was determined). EPI Suite estimated a shorter aerobic soil half-life (t1/2= 25 days) and a longer sediment half-life (t1/2= 100 days). However, once it reaches a receiving water body, it predominantly partitions into the water column. No data are available on the effects of photolysis; however, it was reported that streptomycin is stable for hydrolysis in neutral solutions (at 20 °C) and is unstable in both alkaline and acidic conditions. Based on EPI Suite estimates, streptomycin is very highly mobile (Koc = 10 L kg-1). Given the moderate persistence/high mobility and solubility of streptomycin, the chemical is expected to dissipate relatively slowly and at the same time be vulnerable to leaching/run-off.*

Kummerer (2009a) reports that data on streptomycin concentrations in soil following application to growing fruit are unavailable.

Gavalchin and Katz (1994) studied the persistence of seven antibiotics commonly used in animal feed, including streptomycin, in typical agricultural soil (sandy loam). The level of streptomycin incorporated into the soil with manure was 5.6 µg/g. No detectable streptomycin was found in the soil samples following 30 days of incubation at 30, 20, or 4 degrees Celcius. However, the addition of manure or sludge to soil, such as in this study, has often resulted in increased biodegradation of antibiotics in soil (Thiele-Bruhn, 2003). Furthermore, the extent and kinetics of antibiotic degradation in soil is highly dependent on temperature, soil type, and antibiotic adsorption to soil.

Gardan and Manceau (1984) reported that no surface residue of streptomycin was detectable on pear or apple trees after four to six weeks following spray application. However, Mayerhofer et al. (2009) showed that the use of streptomycin sprays can lead to detectable concentrations of streptomycin in apples. Streptomycin was detected in 20 of 41 samples from orchards that were treated one to three times with streptomycin sprays. The concentration of streptomycin was highest in the apple cores and skin and ranged from 1.9 to 18.4 µg/kg (equivalent to 0.0019 to 0.0184 ppm, well below the EPA's established tolerance of 0.25 ppm).

The RED for streptomycin and streptomycin sulfate concluded that there are no ecological concerns from the use of this naturally occurring antibiotic (EPA, 1992). As part the current registration review for streptomycin, the EPA has called for environmental fate data to determine the persistence of streptomycin in the environment as well as the potential for antibiotic resistance to transfer from plant pathogens in the environment to human pathogens (EPA, 2009). EPA's final registration review decision for streptomycin is scheduled for 2014. The topic of antibiotic resistance as it relates to the use of streptomycin as a pesticide will be discussed in more detail in the response to Additional Question #1 (below).

Based on the limited data available, there is no evidence to suggest substantial, long-term persistence of streptomycin in the environment following its use as a pesticide to control fire blight in apples and pears.

<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)).

Streptomycin helps to control fire blight by killing the bacterial pathogen *Erwinia amylovora*. When streptomycin enters the cells of *Erwinia amylovora*, it binds to cellular components called ribosomes and reduces their ability to correctly synthesize proteins needed for growth and survival. The result is accumulation of erroneous proteins within the cell and cell death (Hermann, 2007).

Animal studies have been conducted with streptomycin to determine the potential toxic effects of this substance (EPA, 1992). Streptomycin was found to have low acute toxicity when administered to rats and mice. A 2-year feeding study in rats indicated that streptomycin does not cause cancer in these animals. No developmental effects were seen when pregnant rabbits were administered streptomycin on the critical days of gestation. Streptomycin sulfate exhibited negative to weakly positive results in a series of genetic toxicity tests to determine its potential to interact with DNA or damage chromosomes – indicating that it is unlikely to cause cancer (NTP, 2005).

The toxicity of streptomycin to humans has been extensively reviewed because of its use in medicine. HSDB (2002) summarizes the toxic effects of streptomycin. Such effects include ototoxicity (hearing loss or vestibular problems), nephrotoxicity (manifested as increased or decreased frequency or urination or amount of urine, increased thirst, loss of appetite, nausea, vomiting), effects on vision, peripheral neuritis (burning of face or mouth, numbness, tingling), neurotoxicity (muscle twitching, numbness, seizures, twitching), and hypersensitivity/allergic reactions (rashes, hives, swelling, anaphylactic shock). The FDA has categorized streptomycin as pregnancy category D due to the risk of fetal ototoxicity (deafness). Pregnancy category D is for substances that have demonstrated positive evidence of human fetal risk, and should only be given in pregnancy when the benefit outweighs the risk. Although there is a risk of fetal deafness following therapeutic doses of streptomycin, the exposure that occurs from the use of streptomycin as a pesticide is not expected to pose this risk. The typical therapeutic dose of streptomycin is 15 to 30 mg/kg body weight, and there is a risk of fetal deafness at this dose. EPA (2006a) has established that chronic exposure to 0.05 mg/kg body weight per day of streptomycin is expected to be safe without risk of adverse effects such as fetal deafness. EPA (2006a) estimated the aggregate exposure to streptomycin due to its use as a pesticide (coming from food, water, and residential uses) and found it to be well below the safe exposure level.

Streptomycin can be phytotoxic at concentrations much higher than those used for control of fire blight in apples and pears. At the appropriate concentrations, it is non-toxic to plants. EPA determined that streptomycin is practically non-toxic to birds, freshwater invertebrates, and honey bees, and is slightly toxic to cold and warm water species of fish (EPA, 1992). Streptomycin is toxic to algae, with cyanobacteria being more sensitive than green algae (Qian et al., 2010). Streptomycin causes toxicity to algae by inhibiting cell growth and photosynthesis-related organelles and proteins. Because of its toxicity to algae, EPA requires that all pesticide products containing streptomycin, except those specifically used as algicides in ornamental aquaria and ponds, include a warning not to apply directly to water or in areas where surface water is present, and not to contaminate water during cleaning of equipment or disposal of wastes.

No information could be found to suggest that agricultural streptomycin products contain toxic contaminants or that the degradation products of streptomycin would result in toxic effects to humans or the environment. As stated in the response to Evaluation Question #4, there is no evidence to suggest substantial, long-term persistence of streptomycin in the environment following its use as a pesticide to control fire blight in apples and pears.

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

No current information could be found on the possible environmental contamination resulting from the manufacture of agricultural streptomycin. The following information was included in the 2006 Technical Report for Streptomycin:

Dzhedzhev et al. (1975) reported that the manufacture of streptomycin resulted in high atmospheric concentrations of the solvents butyl alcohol and butyl acetate in the workplace. In 1998, EPA revised its water effluent limitations guidelines and standards for the pharmaceutical manufacturing industry to control water pollution discharged from these facilities (EPA 1998). Based on information EPA collected from 244 facilities, fermentation operations may use solvents to isolate the substance from the broth and other impurities. Usually, the solvents are recovered and reused, but small amounts of the solvents may remain in the broth "washes" that are discharged in the plant's wastewater. The solvents most frequently used in fermentation operations according to the data collected include acetone, methanol, isopropanol, ethanol, amyl alcohol, and methyl isobutyl ketone. Specific information for the production of streptomycin was not provided, so it is unclear whether manufacturers of streptomycin actually use solvents. Other pollutants that could be discharged from pharmaceutical fermentation processes include detergents and disinfectants used to clean equipment. Nitrogen and sulfur oxide gases may be produced by the fermenters, which are regulated by EPA. Assuming streptomycin manufacturers comply with applicable water and air regulations, it is unlikely that environmental contamination will result from fermenting processes. The *Pollution Prevention and Abatement Handbook: Pharmaceuticals Manufacturing* (IFC 1998) also provides a general discussion of environmental pollution and opportunities to diminish pollution associated with the manufacture of pharmaceuticals, including antibiotics such as streptomycin. No other specific information was found on the potential for environmental contamination resulting from the manufacture of streptomycin.

As stated in the response to Evaluation Question #4, Gardan and Manceau (1984) reported that no surface residue of streptomycin was detectable on pear or apple trees after four to six weeks following spray application. Furthermore, EPA (1988) concluded that streptomycin residues are non-detectable [< 0.5 ppm (parts per million)] on crops when treated according to label use rates and directions. EPA (1988, 1992) cited data that showed that streptomycin biodegrades relatively quickly in soil and water. The breakdown products include methylamine, carbon dioxide, and urea, all of which occur naturally in the environment. Therefore, the application of streptomycin for control of fire blight in apples in pears in accordance with labeled instructions is unlikely to contaminate the environment.

No current information could be found on environmental contamination resulting from misuse or disposal of agricultural streptomycin products.

Because streptomycin is unstable when heated and does not persist in the soil, disposal by incineration or burial should not result in harm to the environment (HSDB, 2002). Streptomycin is toxic to algae and therefore EPA requires that all pesticide products containing streptomycin , except those specifically used as algicides in ornamental aquaria and ponds, include a warning not to apply directly to water or in areas where surface water is present, and not to contaminate water during cleaning of equipment or disposal of wastes.

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)).

The HSDB (2002) states that streptomycin should not be applied following Bordeaux mixture and it is incompatible with lime sulfur, both of which are substances permitted for use in organic crop production. No further information could be found on known chemical reactions between streptomycin and other substances used in organic crop or livestock production or handling.

There is evidence to suggest that some producers are applying streptomycin in combination with tetracyline to apple or pear trees when streptomycin-resistant strains are present in the orchard (Johnson, 2010). No chemical interactions are expected to occur between these two antibiotics.

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)).

Although streptomycin, as an antibiotic, is toxic to some microorganisms in the soil, it is already present in soil due to production by naturally occurring bacteria. Thiele-Bruhn (2003) reported that, in general, the effects of an antibiotic on soil organisms are essentially influenced by the bioavailability of the antibiotic, which depends on soil properties, availability of nutrients, and presence of root exudates.

Ingham and Coleman (1984) demonstrated in a laboratory experiment that application of streptomycin at a rate of 1 mg/g soil did not have a significant effect on total bacteria, fungi, or protozoa counts in soil for 22 days after application. The ammonium-Nitrogen concentration was significantly increased following application of streptomycin, possibly indicating that nitrifying bacteria were susceptible to this bactericide. However, a corresponding decrease in the nitrate-nitrite Nitrogen concentration was not observed. This study also found that application of streptomycin at a rate of 3 mg/g soil caused a continuing reduction in the total bacterial population which lasted longer than the study (22 days). Streptomycin applied at 3 mg/g soil also reduced active hyphae only on the first day following application. The soil used in this study was sterilized soil from northeastern Colorado (semi-arid climate) which was inoculated with bacteria, fungi, and protozoa. No nematodes, arthropods, or plants were present.

According to Kumar et al. (2005), a broad-spectrum antibiotic like streptomycin would be expected to inhibit the nitrification process in soil.

Popowska et al. (2010) demonstrated in a laboratory experiment that the presence of streptomycin in three different types of soils affected the ecological balance in the soil, causing the elimination of some bacterial populations. In this study, varying concentrations of streptomycin (1 – 7 µg/g) were added to three different soil types in a laboratory setting: forest soil from a pine forest, fertile arable agricultural soil, and garden compost. The soils were then incubated for 14 days. The authors found that 2 µg/g and higher concentrations of streptomycin caused a significant reduction in bacterial count and many bacterial species were eliminated from the soils. The eliminated species were described as beneficial bacteria involved in various metabolic processes, mineralization of organic compounds, degradation of toxic compounds, or creating soil structure. This study also isolated from the soils many strains of bacteria demonstrating resistance to streptomycin, including opportunistic pathogens of humans and/or animals.

Kumar et al. (2005) reported that the potency of streptomycin declined over time in experiments using both aerobic and anaerobic conditions in activated sludge and selected soil bacteria. This suggests that the degradation products of streptomycin lack antimicrobial potency.

Based on the limited data available, it is still unclear if the use of streptomycin for control of fire blight has significant negative effects on interactions in the agro-ecosystem, including soil organisms. There are no studies available in the field, and studies in the laboratory with soil bacterial populations appear to be contradictory. Furthermore, no information was found regarding potential effects on the Salt Index and solubility of the soil, earthworms, mites, grubs, nematodes, pH levels, nutrient availability, or endangered species

Evaluation Question #9: Discuss and summarize findings on whether 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)).

The RED for streptomycin concluded that agricultural streptomycin products, labeled and used according to EPA regulations, will not pose unreasonable risks or adverse effects to the environment (EPA, 1992). However, as part of EPA's current registration review of streptomycin, new data are being called for to complete an updated ecological and endangered species risk assessment. These data include environmental fate data to determine the persistence of streptomycin in the environment, avian reproduction data, freshwater invertebrate life cycle data, freshwater fish early life stage data, terrestrial plant toxicity data, and aquatic plant toxicity data (EPA, 2009). The registration review is scheduled to be complete in 2014.

Streptomycin is moderately persistent in aerobic soil. The limited available data suggest that long-term persistence of streptomycin in the environment is not likely to be a concern. Streptomycin is toxic to algae, however risk mitigation in the form of warnings on product labels should prevent significant adverse effects on algal populations in the environment. Manufacture of streptomycin may release solvents, disinfectants, detergents, gases, and streptomycin itself into the environment. Assuming streptomycin manufacturers comply with applicable water and air regulations, it is unlikely that environmental contamination will result from the manufacture of streptomycin. There is a high probability that streptomycin resistant bacteria are present in the environment as a consequence of pesticidal use of streptomycin (EPA, 2006a). This topic is discussed in more detail in the response to Additional Question #1 (below).

<u>Evaluation Question #10:</u> 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)).

The TRED for streptomycin concluded that "there is reasonable certainty that no harm to any population subgroup will result from exposure to streptomycin" (EPA, 2006b).

Current tolerances (maximum residue limits) for streptomycin on or in apples and pears is 0.25 ppm. Assuming that the maximum amount of streptomycin residues are present in all types of food which may contain residues, EPA determined that chronic aggregate dietary exposure from streptomycin residues in food and water is not considered to be a human health concern (EPA, 2006a). Exposure to streptomycin through residential use and/or pharmacological uses in addition to chronic dietary exposure is also not considered a human health concern.

Workers may be exposed to streptomycin while applying products containing this pesticide or while working in fields where crops have recently been treated. The HED Chapter of the TRED states that there have been few reports of adverse effects resulting from use of streptomycin as a pesticide (EPA, 2006a). In one incident reported in California, ten field workers reported allergic reactions and/or itchy sensations, nausea, and headaches following inadvertent exposure to streptomycin spray. Nine other incidents in California involved reports of skin rashes and eye effects mostly in workers exposed to streptomycin residues, as opposed to handlers or mixers/loaders. In order to mitigate the risk to workers, personal protective equipment is advised to prevent skin contact with streptomycin. Furthermore, workers are not permitted re-entry into treated areas for at least 12 hours.

There is a possibility of human exposure to streptomycin resistant bacteria resulting from the use of streptomycin as a pesticide. The human health risks resulting from this exposure are uncertain (EPA, 2006b). This topic is discussed in more detail in the response to Additional Question #1 (below).

<u>Evaluation Question #11:</u> 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)).

<u>Natural (non-synthetic) substances or products:</u>
Biological control agents – Various antagonistic organisms have been studied for use in control of fire blight in apples and pears. The premise of biological control agents (such as bacteria or yeast) is that they are used to out-compete the pathogen where it occurs on the blossom. Some also decrease pathogen numbers through antibiosis (production of a substance that inhibits the growth of the pathogen) (Johnson et al., 2009). Organisms that can grow quickly and deprive *E. amylovora* of food or space without causing

disease are helpful for fire blight suppression. Biological control agents are recommended to be applied to flowers at early bloom (15 to 20% bloom) and at late bloom (full bloom to petal fall) (Sundin et al., 2009). These agents are preventative and must colonize the blossom before infection occurs in order to be effective. Once the antagonistic organisms are established on the stigmas of flowers, warm temperatures (>15 ºC) and pollinator activity will help to ensure colonization and increase the efficacy of the biocontrol agent (Sundin et al., 2009).

All of the commercially available biological control agents are organisms that are indigenous to apple and pear blossoms. Two different strains of the beneficial bacterium *Pantoea agglomerans* have been researched for control of fire blight and registered as the products Bloomtime Biological (Northwest Agri Products, Pasco, WA) and BlightBan C9-1 (Nufarm Americas, Inc., Burr Ridge, IL). The bacterium *Pseudomonas fluorescens* A506 is marketed as BlightBan A506 (Nufarm Americas, Burr Ridge, IL). Two different strains of the yeast *Aureobasidium pullulans* make up the product Blossom Protect (Bio-ferm, Germany) which is currently not available in the U.S. [according to Johnson (2010) this product will be available in the U.S. in 2011 from Westbridge Agricultural Products, Vista, CA]. Other yeast and bacterial strains are being evaluated for use as single antagonists or antagonistic mixtures of *E. amylovora* (Pusey et al., 2009). The product Serenade Max (AgraQuest, Davis, CA) contains a strain of the bacterium *Bacillus subtilis* along with antimicrobial lipopeptides produced during fermentation of this bacterium. The antimicrobial activity of the lipopeptides is thought to be the cause of the product's effectiveness at reducing populations of *E. amylovora* on blossoms (Sundin et al., 2009).

The efficacy of commercially available biological control agents has been widely studied. In one type of field protocol, the antagonistic organisms are sprayed at high doses onto flowers, and then several days later, the flowers are inoculated with a low dose of the pathogen *E. amylovora*. Control plants receive no treatment with antagonistic organisms but are still inoculated with the pathogen. Using this protocol (inoculated fire blight trials), antagonists usually produce only a 40 to 60% reduction in disease incidence when compared to control plants (Johnson et al., 2009). Results have been mixed for the product BlightBan A506. Johnson (2010) describes its effectiveness as poor to fair, stating that it has performed better in field trials with natural pathogen populations as opposed to inoculated trials (also reported in Stockwell et al., 2011). Johnson's summary also reports that strains of *Pantoea agglomerans* (such as Bloomtime Biological and BlightBan C9-1) are usually the most effective biocontrol agents in fire blight suppression, with about a 50% reduction in disease incidence observed in inoculated trials (Johnson 2010). Johnson describes the effectiveness of Bloomtime Biological as poor to good and the effectiveness of both Serenade Max and the European product Blossom Protect as fair to good. By comparison, the antibiotic treatment oxytetracycline is described as fair to very good, and treatment with streptomycin is poor to excellent (the poor rating is due to widespread pathogen resistance to streptomycin within the western states).

Sundin et al. (2009) reports that treatments with BlightBan A506 and BlightBan C9-1 have produced a 40 to 80% reduction in the incidence of fire blight in several trials conducted in the Pacific Northwest of the U.S. However, trials conducted in the eastern U.S. (Michigan, New York, Virginia) have shown that BlightBan A506, BlightBan C9-1, Bloomtime Biological, and Serenade were much less effective in controlling blossom blight when compared to the standard treatment with streptomycin (Agri-Mycin). Management of fire blight is more difficult in the east because of greater rainfall and humidity. A summary of the results from those trials are shown in Table 1.

Stockwell et al. (2011) reports that disease control was more consistent in field trials conducted with compatible mixtures of antagonistic organisms than with single strains. These authors tested strains of *Pseudomonas fluorescens* A506 (similar to BlightBan A506), a mutant strain of *Pseudomonas fluorescens* A506 (extracellular protease-deficient mutant), *Pantoea vagans* C9-1S, *Pantoea agglomerans* Eh252 (similar to Bloomtime Biological), and combinations of these. The treatments were applied to pear trees at 30% and 70% bloom, and then the pathogen was sprayed on the trees at full bloom. The results for biocontrol agents were compared with a control treatment of water. The strain *Pseudomonas fluorescens* A506 decreased disease incidence by only 16% from control on average. The *Pantoea* strains decreased disease incidence by 42 and 55% on average. Combinations of the mutant *Pseudomonas fluorescens* A506 strain with either *Pantoea* strain were more effective (68 and 71% disease reduction on average). Combination treatments with either *Pantoea* strain and the non-mutant strain of *Pseudomonas fluorescens* A506 were not as effective (44 and 59% disease reduction on average). The reason for this difference is that the non-mutant strain degrades a peptide antibiotic which is produced by the *Pantoea* strains. This peptide antibiotic is believed to be a key contributor to the efficacy of *Pantoea* strains against the fire blight pathogen. Antibiotic treatments were also included in these trials for comparisons. Oxytetracycline and streptomycin reduced disease incidence by 39% and 81% on average, respectively.

Table 1. Efficacy of Different Biological Control Agents and Streptomycin in Reducing the Frequency of the Blossom Blight Phase of Fire Blight in Apple Trees at Three Locations in the Eastern United States[a]

Treatment[b]	Mean % Reduction in Blossom Blight[c]
Pseudomonas fluorescens A506 (BlightBan A506)	9.1% (12.5% with the addition of the surfactant Break-Thru)
Streptomycin (Agri-Mycin)	61.0%
Pantoea agglomerans C9-1 (BlightBan C9-1)	33.1%
Pantoea agglomerans C9-1 plus *Pseudomonas fluorescens* A506	26.5%
Streptomycin (Agri-Mycin)	60.4 - 63.3%
Pantoea agglomerans E325 (Bloomtime Biological)	28.5%
Streptomycin (Agri-Mycin)	67.3%
Bacillus subtilis QST713 (Serenade)	36.1%
Streptomycin (Agri-Mycin)	65.9%

[a] Source: Sundin et al., 2009

[b] All field experiments included a control treatment (nontreated, pathogen-inoculated trees). Treatments with formulated biological control agents were typically applied both early in the bloom period and later in the bloom period. Application timings for Serenade formulations and Agri-Mycin were generally at full bloom.

[c] Comparison of frequency of blossom blight in treatment groups versus frequencies in concurrent nontreated control groups.

Cao et al. (2010) of the Ohio State University have provided efficacy ratings for some of the biocontrol agents available for control of fire blight. These ratings are based on the results of one-year field studies published between 2000 and 2009 in the Plant Disease Management Reports (http://www.plantmanagementnetwork.org/pub/trial/pdmr/). The ratings are for each product were determined from a comparison between untreated controls and the application of each product individually. The product Bloomtime Biological was rated as "±" for fire blight control in apples and pears, meaning that evidence for disease control is mixed with some reports showing positive results and others not. The product Serenade Max was rated poorly corresponding to "no obvious response to treatment in one or more published reports." No other biocontrol agents currently used in the control of fire blight were rated by Cao et al. (2010).

Kunz et al. (2008a) describes the results of field trials with the product Blossom Protect conducted on apple and pear orchards in Germany. Treatment with Blossom Protect resulted in an average efficiency of 82% reduction in fire blight incidence (results from six different trials). In each trial, Blossom Protect was applied to plants four times during bloom (this is twice the number of treatments typically applied for fire blight control). After the first application, one tree per plot was inoculated with the pathogen, *E. amylovora*. After that, the pathogen was reported to spread over the entire orchard by natural vectors. Results of disease incidence were only recorded for plants that were not inoculated. Johnson (2010) reports that he and his colleagues evaluated Blossom Protect in an inoculated fire blight trial in 2008 (also using four applications during bloom). They found this product to be nearly as effective as streptomycin (Agri-Mycin) in an orchard with high disease pressure.

As demonstrated by the data presented above, the results are mixed for biological control agents in the suppression of fire blight. While most controlled trials have shown some degree of reduction in disease incidence, the agents usually do not perform as well as streptomycin. However, research is ongoing to find new antagonistic organisms and combinations of antagonistic organisms that provide higher efficacy in suppression of *E. amylovora* (Pusey et al., 2009; Sholberg and Boulé, 2008; Johnson, 2010). Currently, inconsistent efficacy discourages many producers from using biocontrol agents in the fight against fire blight (Stockwell et al., 2011).

Fire blight prediction models – These computer models are based on weather patterns and can be useful in helping the grower decide when to apply a biological control agent. The two most popular models are MaryBlyt© developed by Paul Steiner and Gary Lightner at the University of Maryland and Cougarblight developed by Timothy Smith at Washington State University. These models are also widely used by growers to decide when to apply antibiotic treatments (streptomycin or oxytetracycline).

<u>Allowed synthetic substances:</u>
The following substances are included on the National List and are used for control of fire blight in apples and pears:

- Copper mixtures, including Bordeaux mixture (copper sulfate and lime)
- Peracetic acid
- Tetracycline (oxytetracycline)

Products with copper as the active ingredient can be applied during dormant periods up until the green tip stage. If applied to apples and pears during the blossom period and later, copper may cause phytotoxicity and russeting of the fruit (Smith, 2010). The efficacy of copper products has been described as satisfactory to insignificant. Smith (2010) reports that copper products have not performed well in fire blight trials performed by Washington State University. The level of control when applied to open blossoms varied from 20 to 40%, and the level of control when applied pre-bloom (as recommended to prevent phytotoxicity) was reported to be insignificant. Smith (2010) concludes that copper products are not reliable under conditions of high disease pressure._Adaskaveg and Gubler (2002) report that control of fire blight with copper products is only satisfactory when the threat of disease is low to moderate. No recent trials testing the efficacy of copper products in control of fire blight could be found in the published literature.

Peracetic acid is an oxidizing agent that kills bacteria upon contact. No information could be found on the efficacy of peracetic acid in control of fire blight.

Tetracycline is an antibiotic used to treat fire blight especially in regions of the U.S. where streptomycin-resistant bacteria are common. The level of fire blight control in apples and pears with tetracycline has been reported to be about 40%, which is about half that of streptomycin (Stockwell et al., 2008). Johnson (2010) reports the effectiveness of tetracycline as "fair to very good."

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

Using resistant varieties of apple and pear trees is the most effective prevention method for fire blight (Koski and Jacobi, 2009). Although no cultivar is completely immune to fire blight, some are less susceptible than others. Both Koski and Jacobi (2009) and a report put forth by the University of Illinois Department of Crop Sciences (2005) list the relative susceptibility of common apple and pear cultivars and rootstocks to fire blight. Unfortunately, most of the cultivars demanded by consumers are highly or moderately susceptible to fire blight.

Because fire blight infestation is greatly favored by the presence of young, succulent tissues, cultural practices that favor moderate tree growth are recommended. Such practices include keeping the soil well-drained and limiting or excluding the use of manure (Sholberg and Boulé, 2008; University of Illinois, Department of Crop Sciences, 2005). In addition, careful pruning, disinfection of all tools used in pruning, and/or pruning during the winter when lower temperatures render the bacteria inactive can help prevent spreading the disease from infected to uninfected trees. Smith (2010) states that many organic growers successfully use the blossom removal method to prevent secondary bloom fire blight in pears and apples. This only works in areas of the country where apple and pears trees bloom more than once in a growing season. This method involves removing secondary blossoms by hand when the conditions suggest a high risk of fire blight infection.

Despite following all of these recommendations, fire blight can still devastate an apple or pear orchard. Therefore, biological antagonists and chemical control methods remain important.

Additional Questions Specific to Streptomycin

The following additional questions were posed by the NOSB Crops Committee to aid the National List review for streptomycin use in organic crop production (USDA, 2011).

Additional Question #1: Describe any new evidence on use of antibiotics in crop production; for example is there evidence of contribution to bacterial antibiotic resistance by use of antibiotics as crop pesticides or any evidence of impact on soil organisms?

There is very little information available on the potential negative effects of using antibiotics in crop production. Human bacterial pathogen resistance to streptomycin resulting from its use as a drug has been recognized for many decades. Many uncertainties still remain in regard to pesticidal contributions to antibiotic resistance (EPA, 2006b). Streptomycin remains important in modern medicine, and an increase in streptomycin-resistant bacteria in the environment and in humans may lead to adverse human health consequences. Streptomycin is used today in medicine in combination therapy to treat tuberculosis (due to increasing resistance to other anti-tubercular drugs) and enterococcal endocarditis (when there is resistance to gentamicin). It is also used to treat the plague and tularemia, however there are alternative drugs available to treat those diseases.

The HED Chapter of the TRED for streptomycin includes a qualitative assessment of pesticidal uses of streptomycin contributing to antibiotic resistance of human bacterial pathogens (EPA, 2006a). The data were insufficient to complete a quantitative assessment. The assessment concluded that dietary residues of streptomycin are so low that antibiotic resistance resulting from food exposure is not likely. However, bacterial resistance to streptomycin as a result of pesticidal use has the potential to cause adverse public health consequences if human bacterial pathogens are present in orchards and develop resistance or if non-pathogenic bacteria in orchards develop resistance and later transfer the resistance to human bacterial pathogens. The assessment concluded that "the possibility of antibiotic resistance resulting in adverse human health consequences was of medium concern following occupational application and was of high concern following application by residential users" (EPA, 2006a, pg. 3).

As part the current pesticide registration review for streptomycin, EPA has called for environmental fate data to determine the persistence of streptomycin in the environment as well as the potential for antibiotic resistance to transfer from plant pathogens to human pathogens (EPA, 2009). EPA's final registration review decision for streptomycin is scheduled for 2014.

Rezzonico et al. (2009) state that prohibitions and restricted uses of antibiotics in agriculture have occurred in other countries due to concerns over horizontal transfer of resistance genes from bacteria in the agricultural setting to clinically relevant bacteria. However, such a link has never been documented.

The evidence of pesticidal use of streptomycin impacting soil organisms was reviewed in the response to Evaluation Question #8. Based on the limited data available, it is still unclear if the use of streptomycin for control of fire blight has significant negative effects on soil organisms. There are no studies available in the field, and studies in the laboratory on soil bacterial populations appear to be contradictory. Furthermore, no information was found regarding potential effects on earthworms, mites, grubs, or nematodes.

<u>Additional Question #2:</u> What progress has been made on alternatives, and how are European and Canadian producers or suppliers managing to produce organic apple and pear crops without the allowance for use?

The progress that has been made on non-antibiotic alternatives to streptomycin has largely focused on biocontrol agents. The available research on these agents was described in the response to Evaluation Question #11. In summary, it appears that the commercially available biocontrol agents can be effective at suppressing the causative agent of fire blight (*E. amylovora*), however work is ongoing to determine the best combinations of agents and the most effective timing for applications (Johnson, 2010). Currently, inconsistent efficacy discourages many U.S. producers from using biocontrol agents in the fight against fire blight (Stockwell et al., 2011).

Streptomycin is not specifically listed for use in organic farming by the Canadian General Standards Board or the European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008. From the available information found on the internet, it appears that Canadian organic apple and pear producers are managing fire blight through the use of disease resistant cultivars, biocontrol agents (Serenade Max, Bloomtime Biological FD, BlightBan C9-1 and Blightban A506), Bordeaux mixture (copper sulfate plus hydrated lime), fire blight prediction models, and the cultural methods described in the response to Evaluation Question #12 (Braun and Craig, 2008; Hope-Simpson, 2010; British Columbia Ministry of Agriculture, 2010). Plant extracts have been studied for their effectiveness at suppressing *E. amylovora*. Canadian researchers Sholberg and Boulé (2008) found that sea buckthorn juice produced inconsistent results in screenhouse trials with potted apple trees. In one trial it was as effective as streptomycin, however no other information could be found on sea buckthorn juice.

European organic apple and pear producers appear to be using the same methods as Canadian producers to control fire blight. More research was found coming from Europe on biological control agents (Kunz et al., 2008a; Kunz et al., 2008b; Broggini et al., 2005). The European Union's hard stance against the use of antibiotics in horticulture has led to much research on alternative treatments, in particular biological control agents (Carter, 2007). As mentioned in the response to Evaluation Question #11, the biocontrol agent Blossom Protect (yeast *Aureobasidium pullulans*) is available in Europe and has been shown to be quite effective in the control of fire blight. Other biological control agents are also available in Europe. Researchers Kunz et al. (2008a) found that the European product Myco-sin produced a 65% decrease in disease incidence in fire blight field trials with apple and pear trees. The authors describe this product as "stone meal," and the manufacturer describes it as containing "Sulphuric Clay, Horsetail Essence" (BIOFA, 2011). No additional information could be found on the use of stone meal for control of fire blight.

<u>Additional Question #3:</u> What reasons do all other countries have for the prohibition on this material?

No information could be found on the reasons why streptomycin is not allowed for organic production in Canada.

Streptomycin is not allowed as a plant protection product in conventional or organic production in the European Union. No specific information could be found on the reasons why streptomycin is not allowed for organic production in the E.U., although the reasons are likely similar to conventional agriculture. The use of streptomycin as plant protection product in conventional agriculture was withdrawn by Commission Decision 2004/129/EC. Antibiotics were removed as plant protection products based upon the risk of cross resistance (the spreading of antibiotic resistance genes to human pathogens) (Kyprianou, 2007). The Commission can grant emergency authorization for plant protection products containing antibiotics in specific circumstances when every attempt has been made to restrict the use of antibiotics.

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