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Colloidal Silver – Use in Kiwifruit

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Potential for Use of Colloidal Silver as a Pesticide to Treat *Psa* in Kiwifruit Vines

Summary

Colloidal silver (nanosilver) is a potent antimicrobial substance. It is effective against Psa, but may also inhibit Psa-specific phage. There is no evidence for any preventative effect prior to infection.

Nanosilver has a number of potentially beneficial applications in health and commerce however there is global consensus the level of information regarding its potential toxicity and long-term effect is lacking.

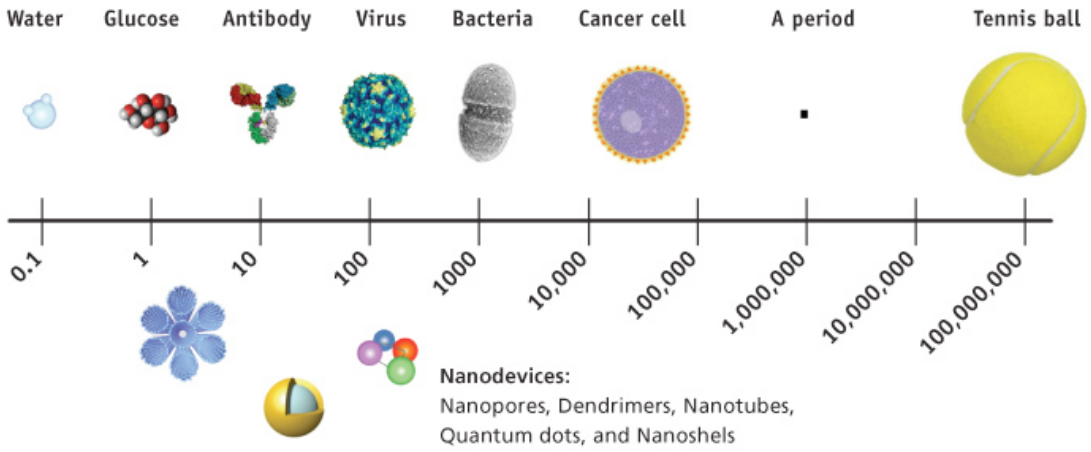
There is growing concern around the world of the potentially harmful effects of the accumulation of silver ions in the environment and the long-term harmful effects on aquatic, terrestrial and plant life.

There is no available data relating to potential accumulation of silver ions in fruit. MRL's do not specifically address silver, as it is not a traditional agricultural compound. Potential limits on silver in foods, is likely to be as a contaminant. Depending on analytical methodology, it may currently contribute to the total heavy metal loading, which is subject to strict enforcement around the world.

In NZ the use of nanosilver (colloidal silver) preparations as a pesticide in kiwifruit is not approved by the HAZNO Act 1996, and any person preparing (manufacturing) such solutions for use is contravening this Act. Similarly there are no registered colloidal silver based compounds under the ACVM Act 1997. There is potential under the ACVM Act for own use of colloidal silver as an antibiotic agent, however this is subject to the a requirement to have an approved operating plan for preparation, use and storage. This plan will include meeting HAZNO requirements.

1. Background

Colloidal silver is prepared through the ionisation of silver metal in de-ionised water, resulting in a suspension of fine silver particles, typically 10-1000nm (Faunce and Watal 2010). Nanosilver is the term used to define silver particles < 100nm, and today the 2 terms are considered synonymous (Silver Nanotechnology Working Group (SNWG) 2009), colloidal silver being one of a number of forms of nanosilver.



http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm153723.htm (accessed 9 November 2011)

Nanosilver (in the form of colloidal silver) has a long history of use (>120 years), and was first registered as a pesticide in the USA in 1954 (Varner, Sanford et al. 2010). Nanosilver has along history of use related to its potent antimicrobial functionality; however interestingly there is a relative dearth of historical scientific data directly relating to its toxicity, and mode of action. The mode of action as an antimicrobial is based on its toxicity to bacterial, viral and fungal cells. It is used as a treatment to kill microbes post infection or contamination – not as a preventative or deterrent measure. In recent times with the rapid development of nanotechnology and a greatly expanding range of applications for nanosilver, research into the potential adverse effects of increasing silver levels in the environment and food chain are now receiving attention. Faunce and Watal (2011) argue that whilst the wide range of medical devices, therapeutic products and domestic food and goods containing nanosilver offer numerous benefits, such applications need to be subject to precautionary regulation. With increasing human exposure to nanosilver, and the increasing environmental levels of silver there is growing concern for long-term toxicity (human and environmental) issues.

1.1. **Human Health**

Nanosilver has a long history of human use, from an original application as a neonatal ophthalmic solution to protect against maternal gonorrhoea, through to use during WW1 in wound dressings and as a disinfectant. The development of antibiotics superseded the use of silver by the late 1940's (Faunce and Watal 2010). Today nanosilver is used in an extensive range of medical devices (burn & wound dressings, prosthetics, catheters, implantable devices, surgical and dental instruments to prevent infection and sepsis. Some evidence is emerging to suggest migration of the nanosilver into patients (Varner, Sanford et al. 2010).

Silver itself is not considered toxic, however its salts are, and in the ionised state it is highly active at the cellular level.

Chronic ingestion of nanosilver (as colloidal silver solution) results in argyria, a permanent discolouration (slate grey) of the skin. Although silver is generally considered benign, argyria has been associated with neurological, renal, optic and hepatic dysfunction (Kwon, Lee et al. 2009). There remains some conflicting evidence as to the toxicity of nanosilver solutions, with acute and subchronic toxicity testing in mice and guinea pigs indicating no signs of toxicity in short term administration of colloidal nanosilver (Maneewattanapinyo, Banlunara et al. 2011)

Recent toxicological studies on nanosilver suggest a range of adverse metabolic and histopathological effects, target organs being the skin, liver and spleen (Korani, Rezayat et al. 2011). There is mounting evidence nanosilver can penetrate biological barriers and enter cells, disrupting fundamental cellular processes resulting in the generation of reactive oxygen species (ROS), disrupting redox cycling, deactivating enzymes and generating DNA damage (Priestly 2011). The very mechanisms that contribute to its potent antimicrobial properties may be equally toxic to healthy cells.

The use of nanosilver in public health applications such as the purification of drinking water are viewed as potential benefits to human health in developing nations or emergencies.

In the USA OTC silver preparations for the treatment of any condition require FDA approval (21 CFR Ch I 310.548) due to the concerns of the FDA, and lack of adequate data to establish their general safety and effectiveness. A 2008 petition to EFSA for nanosilver also failed to reach the assessment stage due to a lack of toxicological data (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) 2008). The potential for bacteria to develop resistance to silver treatments also is not known (Chopra 2007).

In summary the mechanisms and human toxicity exposure data are not well understood or established. Exposure to silver in air should be less than 0.010mg/m3, and in 1991 the US EPA established an oral reference dose of 0.005mg/kg/day for silver (Varner, Sanford et al. 2010).

1.2. **Commercial Applications of Nanosilver**

In addition to the direct applications for human health there is an increasing number of uses of nanosilver in consumer products (clothing, appliances, domestic utensils, sanitisers, wipes to mention a few) capitalising on its potent antimicrobial properties. The range of scientific, industrial and electronic applications of nanosilver continues to increase rapidly.

1.3. **Is there concern?**

In short YES. The technological benefits of nanosilver have led to its burgeoning use. Nanosilver has a propensity to leach into, and accumulate in, the environment, where it continues to exhibit potency. Its accumulation in the environment and eco-toxicity are significant concerns within regulatory agencies worldwide.

2. **Regulatory Status of Nanosilver in New Zealand**

2.1. **Food Standards – Standard 1.4.1 Contaminants and Natural Toxicants.**

This Standard sets the maximum levels for specified metal and non-metal contaminants in nominated foods. Metal contaminants are specified and do NOT include silver.

MRL's for pesticides and chemicals are not set within the Food Standards Code for NZ, however the Code does cover Australia.

For Australia there are no MRL's set for silver.

2.2. **Hazardous Substances and New Organisms Act 1996**

No silver substances are listed or registered as pesticides in NZ. The use of nanosilver as an injectable substance in plants to combat *Psa* means its use is primarily that of a pesticide.
Under the HAZNO Act any substance deemed to be hazardous requires an application to the Environmental Protection Agency (Section 5 Caluse 28) prior to manufacture. Given the level of concern regarding nanosilver and its potential eco-toxicity, EPA would consider nanosilver (colloidal silver) to fall within the scope of hazardous substances and therefore require approval based on application (*pers. comm.* H Murdoch EPA). This would apply to farmers "manufacturing nanosilver for use as pesticide".

Approvals for hazardous substances

28 Application for approval to import or manufacture hazardous substances

(1) Unless an approval under section 28A or section 29 applies to the importation or manufacture of the substance, every person intending to—

(a) import; or

(b) manufacture—

a hazardous substance otherwise than in containment shall, before importation or manufacture, apply to the Authority for approval to import or manufacture that substance.

(2) Every application shall be in an approved form and shall include—

(a) the unequivocal identification of the substance and its properties; and

(b) information on all the possible adverse effects of the substance on the environment; and

(c) information on the intended uses of the substance throughout the life cycle of the substance; and

(d) information on methods for disposal of the substance; and

(e) information on all occasions where the substance has been considered by the government of any prescribed State or country or any prescribed organisation and the results of such consideration; and

(f) such other information as may be prescribed.

2.3. **ACVM**

There are no approvals for the use of colloidal or nano- silver under the Agricultural Compounds and Veterinary Medicinces Act 1997 (ACVM) (*pers. comm.* G Bradbury MAF). Within the ACVM Act is a prerequisite for compounds to have been evaluated, approved and comply wth the requriements of the HAZNO Act.

However the ACVM Act (Schedule 2 (2)) does potentially permit the preparation and "own use" of colloidal silver, subject to certain conditions, as detailed in the excerpt table below. As it is being used in an antibiotic active capacity it falls within the scope of this Clause, however it does require an **approved operating plan**.

Furthermore it cannot be sold or distributed for this purpose and does remain subject to other legislation, including HAZNO.

Exemption	Conditions
Substance or compound (not being an agricultural compound described elsewhere in this schedule)— (a) prepared by a person (**person A**) for use on animals or plants owned by person A, or in any land, place, or water owned or occupied by person A (and not for sale); or (b) used by person A, or a person employed or engaged by person A, or another person expressly authorised by person A, as described in paragraph (a)	If the substance or compound is used by a person employed or engaged by person A or another person expressly authorised by person A, the use must be in accordance with written instructions from person A about— (a) how the substance or compound is to be stored, prepared for use, administered, applied, and (if applicable) disposed of; and (b) how the safety and welfare of any person or animal who may come into contact with the substance or compound is to be protected and how any pain or distress of an animal is to be mitigated; and (c) how third parties are to be contacted or advised of the use of the substance or compound and warned of any hazards relating to the use of the compound The following substances or compounds may be prepared or used as described in column 1 only in compliance with an **approved operating plan:** (a) active ingredients that are prescription medicines or restricted medicines (as those terms are defined in the Medicines Act 1981): (b) **antibiotic active ingredients**: (c) hormones: (d) substances that are prohibited by countries importing New Zealand primary produce: (e) vertebrate toxic agents

2.4. **Contaminant or Residue?**

Substances covered by MRL's (residues) are those routinely used as agricultural compounds and this historically does not include silver – hence it is not included in MRL tables.

Metal levels in foods and medicines are typically considered **contaminants.** Again silver is historically not considered a concern due to the low level of exposure and use, and no specific limits have been set by regulatory authorities. However with the growing concerns over increasing use and environmental contamination this may change. These concerns (leaching and contamination) suggest that any potential limits on silver would be as a **contaminant**.

What is of note is that silver will respond and contribute to the total heavy metal load in routine heavy metal analysis (total) using colourmetric methods (e.g. USP231). The USP and Ph. Eur. (American and European pharmceutical standards respectively) specified limit for total heavy metals is 20ppm. More specific test methods such as ICP-MS) (inductively coupled plasma-mass spectrometry) identify the specific metals. The more recent standard (USP232) for requires individual metal quantification, and lists 2 classes:

Class 1: the highly toxic metals (lead (Pb), arsenic (As), cadmium (Cd) & mercury (Hg)).

Class 2: common processing catalysts including chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickle (Ni), palladium (Pd), platinum (Pt), vanadium (V), osmium (Os), rhodium (Rh), ruthenium (Ru), and iridium (Ir).

Table 1. Elemental Impurity Classes

Class	Assessment
Class 1	Elements should be essentially absent
	Known or strongly suspected human toxicants
	Environmental hazards
Class 2	Elements should be limited
	Elements with less toxicity than Class 1
	Elements deliberately added to an article

These metals are nominated for analysis in relation to the manufacture of drug products and limits set are based on EMEA (European Medical Agency) guidelines. Silver is not included as it is not routinely used as a catalyst in drug manufacture.

3. Use of Nanosilver for Plant Protection

There appears to be little published research on the effectiveness of nanosilver as an anti-microbial in crops. There is a plethora of anectodal and commercial information by promoters and manufacturers of nanosilver products that can be found on the web – but it is not linked to scientific articles.

Surface application of a nanosilver (colloidal) solution to cucumbers in a greenhouse environment was found to be effective at controlling the development and spread of downy mildew, without any signs of phytotoxicity (Alavi and Dehpour 2009).

The role of silver ions in the inhibition of ripening of fruit crops (tomatoes) has been investigated (Davies, Hobson et al. 1988). There is a body of research in this field looking at mechanisms of ripening. In seed crops nanosilver has been investigated as an agent to promote seed abscission to enhance seed and hence oil recovery (Seif Sahandi, Sorooshzadeh et al. 2011). Researchers observed differing levels of silver accumulation in various parts of the plants (shoots, leaves, etc).

More recently a study completed by researchers ar Duke Unitversity in the USA found nanosilver may adversley affect plant growth. The silver nanoparticles were added to the soil in biomatter – representative of potential waste matter. The research team found the nanosilver significantly altered plant growth, microbial biomass and microbial soil activity, suggesting the soil microbes have a direct influence on the health of plants (Zeliadt 2011).

4. Silver and *Pseudomonas*

Organisms listed as susceptible to the toxic effects of nanosilver *Pseudomonas spp.* are included (Varner, Sanford et al. 2010).
Interestingly one of the few relevant articles located suggested *Psuedomonas aeruginosa* is capable of developing resistance to heavy metals, however this was not observed for silver - *Psa* remained sensitive at the levels tested (de Vicente, Avilés

et al. 1990). The work of de Vicente *et al.* focussed on Psa in aquatic and marine environments, in relation to the accumulation of heavy metals resulting from pollution.

4.1. **Phages of *Pseudomonas***

In an investigation into the effects of environmental factors on adsotption and inactivation of Pseudomonas aeruginosa-specific phages, silver (in the form of silver nitrate) was found to inactivate phage acitivity and their activity to inhibit bacterial growth and biofilm formation. The phage δ is a good candidate for the biocontrol of *Psa*, however its performance can be affected by environmental contaminants

Alavi, S. V. and A. A. Dehpour (2009). Evaluation of the nanosilver colloidal solution in comparison with the registered fungicide to control greenhouse cucmber downy mildew diseaes in the north of Iran.

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Silver Nanotechnology Working Group (SNWG) (2009). Evaluation of Hazard and Exposire Associated with Nanosilver and Other Nanometal Oxide Pesticide Products. Presentation to the FIFRA Scientific Advisory Panel. Arlinton VA.

Varner, K., J. Sanford, et al. (2010). State of the Science Literature Review: Everything about Nanosilver and More. Scientific, Technical, Research, Engineering and Modeling Support Final Review, U S Environmental Protection Agency.

Zeliadt, N. (2011). Silver beware: Antimicrobial Nanoparticles in Soil May Harm Plant Life. Scientific American.

Summary Sheet: Nanosilver from the Danish Ministry of the Environment EPA (Mikkelsen, Hansen et al. 2011)

Nanomaterial characteristics		
Name	Silver; NanoAg	 Source: http://www.tradevv.com/
CAS number	7740-22-4 (elemental Ag)	
Chemical composition	Ag	
Appearance	White lustrous powder	
Manufacturing processes	Ultra-sonic precipitation, chemical vapour deposition, exploding wire synthesis. The size, shape, surface area, etc. can be modified by adding various surface active agents and coatings to syntheses involving silver salts.	

Nanomaterial description

Nanosilver (AgNP) is the nanoform of silver characterized by being spherical particles of sizes ranging from 1-250 nm. AgNP is commercialized as powder, flakes, grains, ingots, etc., and is sold in suspension (in water, alcohol or surfactant) and as a dry powder. AgNP also available in preparations (e.g. as a coating agent, in alloys, etc.) and in articles (electrodomestic appliances, in textiles, in food packages, etc.). In its pure form AgNP will aggregate and hence nanosilver is often surface modified with for instance dextran, citrate, or PVP. Sometimes AgNP is also found to be deposited on or used as a coating of a substrate such as plastic, silica or polymers, to give a desired adhesion, electrical conductivity, etc. In aqueous solutions AgNP forms dissolved free silver ions in aqueous by dissolution and subsequent oxidation.

Applications

The use of AgNP is very diverse and include therapeutic applications (diet supplement), personal care products, powdered colours, varnish, textile, paper, interior and exterior paints, printing colours, water and air-purification, polymer-based products and foils for antibacterial protection such as washing machines, kitchenware and food storage. The AgNP concentrations used are unknown for most applications. The scale of use of AgNP is unknown at this point in time, but expected to increase rapidly as more and more consumer products with AgNP are entering the market.

Human health risk profile

It has been shown that silver nanoparticles can be absorbed via especially the oral and inhalational route, whereas intact skin is a barrier against absorption. However, it is unclear in which form (as particles, free ions, silver ions or complexes) nanosilver is absorbed and distributed to target organs. At least for uptake via the oral route it is likely that at least some of the uptake occurs as ions. It appears that smaller particles exhibit higher toxicity as compared to larger particles; and if silver is absorbed as particles then the surface area is relevant.

Should silver uptake occur solely as ions, the database for silver could be applied to assess systemic silver nanoparticle toxicity. For that exercise, it would need to be considered whether and how the dramatically increased surface area and possibly increased solubility of silver nanoparticles would need to be taken into account.

Quite a few, mainly *in vitro* studies, have shown that the main mechanism of silver nanoparticle toxicity seems to be mediated by an increase in ROS production, stimulating inflammation and genotoxic events and apoptosis or necrosis. The concentration of the administered nanoparticles is able to influence the toxicity, specifically, at low levels of oxidative stress a protective response is initiated which progresses to a damaging response with increasing particle concentration, and therefore oxidant levels. It is thus relevant to consider that the toxicity of silver nanoparticles is thresholded.

No data are identified about consumer exposure to nano-silver. The most important exposure route for consumers is expected to be dermal exposure from textiles and cosmetics. However, from available data only minimal absorption is expected even on damaged skin, but further *in vivo* studies would result n more precise estimates.

Consumer exposure via inhalation can occur from products used in spray form. Based on available toxicological data, it is recommended to avoid exposure via inhalation, because health effects like reduced lung function and toxic effect on the liver are considered to be the critical effects from inhalation.

It is still unclear whether nano-silver is more toxic than bulk silver after oral exposure, especially after long term exposure. The toxicity of silver after oral exposure is low, however after long term exposure silver can accumulate in the skin and different organs, which can result in a bluish-gray colouring of the skin (agyria) or deposition in the eyes (agyrosis). The effect on other organs after long term exposure is still unknown.

For long term (life time) exposure a limit of 0.01 mg/L is set for silver in drinking water.

Even though the oral exposure is not expected to be the main exposure route for consumers, exposure from tooth paste can occur and might be close to the exposure from drinking water. High exposure could be expected from intake of food supplements containing nano- (colloidal) silver, which are available on the internet, but not approved for use in Europe.

Environmental risk profile

Silver is known to be an ecotoxic metal and test with silver nanoparticles (AgNP) do also reveal very low effect concentrations. Thus, for algae EC_{10}-values as low as 4 μg/L has been found and also for crustaceans values far below 1 mg/L has been reported. This ranks AgNP as "Aquatic Chronic 1". It is also important to note that at concentrations below 1 mg/L inhibition of nitrifying bacteria can occur and thus the function of wastewater treatment plants may be affected by the presence of AgNP. For ionic silver it is known that the speciation in aqueous media is determining for the bioavailability and toxicity. This is most likely also the case for elemental silver nanoparticles, but influence of speciation on uptake, depuration, and toxicity remain to be studied in this case.

The environmental concentration resulting from the use of AgNP in consumer products are at present uncertain, even though a number of different estimates have been proposed. It has been documented that even though silver nanoparticles are incorporated in textiles, they can be released upon washing. Concentrations in the low ng/L range have been proposed and even such low concentrations may under very precautious assumptions[9] constitute an environmental risk due to the high toxicity of silver.

It is debated today whether silver nanoparticles are in fact more toxic that their bulk counterpart, since effects in many cases can be ascribed to the ionic form of silver (Ag^+). Some studies have documented a higher effect of AgNP, but it is the widespread and dispersive use of silver in consumer products that poses the greatest risk to the aquatic and terrestrial environment. Even if AgNPs are "only" as toxic as larger silver particles, silver is still a metal of high environmental concern.