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.

Oxalic acid

Livestock

Identification of Petitioned Substance

Chemical Names:
Oxalic acid (incl. anhydrous and dihydrate forms)
Ethanedioic acid (incl. anhydrous and dihydrate forms)
$C_2H_2O_4$
$(COOH)_2$
$C_2H_2O_4 . 2(H_2O)$
$(COOH)_2 . 2(H_2O)$

Other Names:
OAD
Oxiric acid
Ethanedionic acid
Acidum oxalicum

Trade Names:
Oxalic acid dihydrate
Api-Biocal

CAS Numbers:
144-62-7
6153-56-6 (dihydrate)

Other Codes:
UNII: 0K2L2IJ590
EC: 205-634-3

Summary of Petitioned Use

Oxalic acid has been petitioned for addition to the National List at §205.603 for the control of varroa mites in organic honey bee hives. The petition refers to three EPA-approved application methods: by solution to package bees, by solution to beehives, and by vapor treatment to beehives. All of these uses are included within the scope of this report. This full technical report also addresses additional focus areas requested by the National Organic Standards Board (NOSB) Livestock Subcommittee:

- *Is there a possibility of varroa mites to develop resistance to the use of oxalic acid? See Evaluation Question #8.*
- *Is oxalic acid used in rotation with other products used to control varroa mites, such as formic acid or other natural controls? See Evaluation Question #11.*
- *Are there other management methods available to control varroa mites? See Evaluation Question #12.*

Unless otherwise specified, this report uses the terms "honey bee(s)" or "bee(s)" to refer to the Western honey bee species *Apis mellifera*. The terms "varroa mite(s)" or "mite(s)" are used to refer to the species *Varroa destructor*.

The following abbreviations will be used for oxalic acid: OA, when referring to oxalic acid forms nonspecifically; OAD, when referring specifically to oxalic acid dihydrate (a solid); and AOA when referring to specifically to anhydrous oxalic acid (also a solid). Solutions prepared with OAD will be called OAD solutions. Solutions prepared with AOA will be called AOA solutions. The EPA's registration decision (EPA 2015a) identifies OA by the CAS Number 144-62-7 (corresponding to anhydrous forms) but also refers to OAD by name (which is characterized elsewhere by CAS Number 6153-56-6). As applied, OAD and AOA starting material becomes the same material (either when dissolved in liquid or vaporized); however, the amount of oxalic acid applied can vary depending on the form used. All forms of OA are included within the scope of this report, although OAD is the form used in EPA-approved products.

Characterization of Petitioned Substance

Composition of the Substance:

Oxalic acid (OA) is the smallest dicarboxylic acid, composed of a bonded pair of carboxyl groups (see Figure 1 below) (King and Korter 2010). When solid, it exists in two crystalline forms: anhydrous oxalic acid (AOA) and oxalic acid dihydrate (OAD). Pure OAD is 71.42 wt % oxalic acid and 28.58 wt % water (Riemenschneider and Tanifuji 2011). When dissolved, OA is no longer in a crystalline form and therefore neither anhydrous nor a dihydrate.

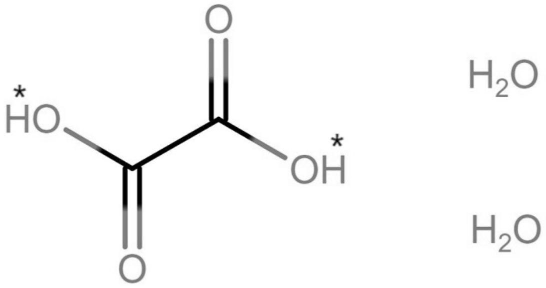

Figure 1: Chemical structure of oxalic acid dehydrate (OAD), adapted from the National Library of Medicine (U.S. National Library of Medicine 2018a). Asterisks note where protons (hydrogen ions) can be lost, forming either an acid oxalate (loss or exchange of only one proton) or an oxalate (loss or exchange of both protons).

Source or Origin of the Substance:

Chemical sources

On an industrial scale, OA is produced as the dihydrate (OAD) via chemical reactions through numerous routes, including oxidation of carbohydrates and other organic compounds using nitric acid (NCBI 2018; Sullivan, et al. 1983; Rohl and Knepper 1975; Riemenschneider and Tanifuji 2011), heating sodium formate in the presence of sodium hydroxide or sodium carbonate (Walter 1996; NCBI 2018; Riemenschneider and Tanifuji 2011), and reacting carbon monoxide with butanol (Fenton and Steinwand 1968; Cassar and Gardano 1978; Riemenschneider and Tanifuji 2011). These manufacturing processes are discussed in detail under *Evaluation Question #2*.

Commercial OA is produced from chemical sources. According to a summary of market data published in 2008, 124,000 tons of OA were produced from chemical sources, with no known microbial sources (Sauer, et al. 2008). China is the leading producer of OA, while the United States is not known to produce any (Kharas 2014). Kharas (2014) states that the annual consumption in the United States (8,000 tons) and annual global consumption (115,000 tons) adds up to a total market value of $1.1 billion per year.

Natural sources

Naturally occurring OA (e.g., in plants, fungi, bacteria, and animals) exists in salt forms such as potassium, calcium, or other oxalates (Riemenschneider and Tanifuji 2011). It can be a considerable constituent of the dry weight of some plants, including beet leaves (12 percent), cocoa (4.5 percent), tea (3.7 percent), spinach (3.2 percent), rhubarb (2.4 percent), and chard (0.69 percent) (Riemenschneider and Tanifuji 2011). It occurs naturally in honey at concentrations between 8–300 mg/kg (Charrière and Imdorf 2002) and is also produced in the human body via the metabolism of glyoxylic acid or ascorbic acid (NCBI 2018).

In microorganisms, OA is produced through two biochemical pathways that oxidize carbohydrates via the citric acid cycle or variants thereof (Kobayashi, et al. 2014). Some organisms may also produce OA to bind and detoxify metals such as aluminum and copper (Hamel, Levasseur and Appanna 1999; Green III and Clausen 2005). In the fungal pathogen *Sclerotinia sclerotiorum*, OA damages plant cells and inhibits plant defense responses (Durman, Menendez and Godeas 2005). Similarly, in the industrially important fungus *Aspergillus niger*, OA may be involved in degrading plant cell walls to mobilize nutrients (Mai, Lee and Choi 2016).

Other sources

OA can exist in the atmosphere from both natural and synthetic sources. It is a component of car exhaust, for instance, but may also form naturally from acetylene or ethene in the aqueous phase of clouds (Crahan, et al. 2004; Warneck 2003). OA is the most abundant dicarboxylic acid in aerosols[1] in the troposphere, ranging from 10-50 ng/m^3 in remote regions to 900 ng/m^3 in urban air (Warneck 2003).

Properties of the Substance:

OA exists as both a solid and as a vapor (i.e., gas). When the solid is heated above its melting point (see Table 1) but below its decomposition temperature, it will sublimate[2] to the gaseous state (Hussain, Khan and Shabeer 2012; Riemenschneider and Tanifuji 2011). The dihydrate solid, OAD, contains two molecules of water of crystallization; dehydrating OAD (e.g., through heating) will yield the anhydrous form AOA (Walter 1996; Riemenschneider and Tanifuji 2011).

OA molecules are arranged in flat sheets or chains within the crystal, with the carboxyl groups oriented in the trans conformation (i.e., facing opposite directions) with respect to each other as shown in Figure 1 on the previous page (Godfrey, Mirabella and Brown 2000). OA crystals will dissolve in polar solvents such as water or alcohol (Hussain, Khan and Shabeer 2012), though alcohols will also partially react with OA to form oxalate esters (Riemenschneider and Tanifuji 2011).

OA is a strong acid that forms metal-oxalate salts (Riemenschneider and Tanifuji 2011). It is dibasic, being able to donate protons (hydrogen ions) from two separate acidic groups. The nearness of the two acidic groups leads to an increase in the strength of acidity; OA has a dissociation constant (K_1) comparable with some mineral acids, including sulfurous acid. The salts are either acid oxalate (i.e., a single cation replacement) or normal salts (i.e., where both acidic groups are replaced with cations) (Walter 1996). OA acts as a reducing agent (American Chemical Society 2006), decomposing into carbon dioxide and water (Riemenschneider and Tanifuji 2011). While OA is soluble in water at neutral pH, oxalate salts such as calcium oxalate are not (with the exception of alkali metal and iron (III) salts) (Hussain, Khan and Shabeer 2012; Riemenschneider and Tanifuji 2011).

Table 1: Properties of the principally used form, oxalic acid dihydrate

Property	Value[a]
Physical state and appearance	Crystalline solid
Odor	Odorless
Taste	Strongly acidic
Color	White or colorless
Molecular weight	126.07 g/mol
Specific gravity	1.65
Bulk density	0.977 g/cm^3 (regular); 0.881 g/cm^3 (coarse)
pH	1.3 (0.1 M solution)
Solubility	14g in 100mL water at 20°C; 40g/100 mL ethanol
pKa	pK_1: 1.27; pK_2: 4.28
Melting point	101°C
Decomposition temperature	157°C
Vapor Pressure	22 hPa (50°C)
Stability	Stable at room temperature / normal conditions
Other	Sublimates readily between 100–157°C

[a]Sources: (American Chemical Society 2006; Walter 1996; LabChem 2018; Science Lab.com 2013; O'Neil, et al. 2013; Hussain, Khan and Shabeer 2012; Riemenschneider and Tanifuji 2011)

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[1] An aerosol is defined by *A Dictionary of Physics* as: *"A colloidal dispersion of a solid or liquid in a gas"* (Law and Rennie 2015). In other words, aerosols are small airborne droplets or particulates.

[2] Sublimation is defined by *A Dictionary of Physics* as: "A direct change of state from solid to gas" (Law and Rennie 2015). Materials that sublimate do not transition to a liquid phase in the process of melting.

Specific Uses of the Substance:

Uses in honey bee production

OA is used in honey bee production to control varroosis, a disease caused by the parasitic mite *Varroa destructor* (CABI 2017, Rosenkranz, Aumeier and Ziegelmann 2010). The mite parasitizes many species of bees, including *A. mellifera* and its subspecies, in both the adult and brood life stages (CABI 2017; Adjlane, Tarek and Haddad 2016). The mites feed directly on honey bees, transmitting diseases and increasing bees' susceptibility to pathogens and bacterial infections (Rinkevich, Danka and Healy 2017). A parasitized brood results in mortality or deformation in surviving nymphs (Adjlane, Tarek and Haddad 2016).

OA is applied to bees in hives through three principle modes: spraying, sublimation (commonly referred to as "vaporization"), and trickling (Rademacher, Harz and Schneider 2017). The use of cellulose strips impregnated with solutions of OA which are mounted on the top bars of hive combs has also been investigated as a hive treatment (Maggi, et al. 2015).

In *spraying*, OA and sucrose are dissolved in water and the solution is sprayed directly onto bee packages or bees in a hive, on both sides of each comb and on the hive walls (EPA 2015a; Rademacher and Harz 2006). Application normally occurs during the broodless period[3] because the treatment is not effective at killing mites in sealed brood cells (Rademacher and Harz 2006) and it can harm or kill bee broods (Rademacher, Harz and Schneider 2017).

In *sublimation*, also known as the vaporizer method, gelatin capsules or tablets containing OAD are heated, causing the OAD to sublimate (Rademacher and Harz 2006). The oxalic acid gas condenses on the bees' bodies in crystal form where it is active against the varroa mite (PMRA 2010). Prior to treating outdoor colonies, cracks in the hive are sealed, and the vaporizer apparatus is inserted into the hive's restricted lower entrance (EPA 2015a). As with the other methods, sublimation treatments are performed during the broodless period. One of the noted disadvantages of this method is the complexity of the vaporization equipment required (Nanetti, Büchler, et al. 2003; Rademacher and Harz 2006).

To simplify the application of OA and minimize disturbance to bees, researchers in Europe developed the *trickling* technique in the 1990s (Nanetti et al. 2003). Using a syringe, solutions of OA dissolved in water (with optional additions of sucrose or glycerol) are trickled directly onto comb top bars or in between frames during broodless periods, often in the autumn (Nanetti et al. 2003; Rademacher and Harz 2006) (Rademacher and Harz 2006). The use of OA trickling during summer in breeding colonies has shown reduced efficacy (Shuster and Schürzinger 2003, reported in Rademacher and Harz 2006) and likely requires more repeat applications than in autumn, where applications are often repeated every seven days for up to four consecutive weeks (Rademacher and Harz 2006). The trickling method has become the favored method for OA application in honey bee hives due to its simplicity and minimal disturbance to the hive (Nanetti, Büchler, et al. 2003).

Experimentally, OAD is used in different concentrations and dosages ranging from 2.1–5 percent OAD in syrup solutions of 0–60 percent sugar (Nanetti, Büchler, et al. 2003; Adjlane, Tarek and Haddad 2016; Mutinelli, et al. 1997; Rademacher and Harz 2006). Doses have been reported as approximately 0.4 mL per dm^2 (Nanetti, Büchler, et al. 2003) and 5 mL per seam (i.e., the space between combs occupied by bees) (Adjlane, Tarek and Haddad 2016; Mutinelli, et al. 1997; Rademacher and Harz 2006). In Canada, recommended application rates are 50 ml solutions containing 35 g OAD in one liter of a 1:1 sugar-to-water (weight:volume) ratio per hive, equivalent to 1.75 g OAD per hive (ATTTA 2017). Commercial EPA-registered products instruct users to apply this same concentration and dose using the solution (trickle)

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[3] The brood rearing cycle (i.e., the pre-adult bee life stages from egg to pupae that occur in specialized hive cells) is affected by factors such as day length (latitude), climate, availability of flowers and nectar, and bee genetics (Crane 1990). In temperate latitudes, brood rearing may cease naturally in late fall. Periods when bees are not rearing brood are referred to as "broodless periods," which can also be induced by physically isolating bee queens (Gregorc, Alburaki, et al. 2017).

method, and to use 1.0 g OAD powder per brood chamber for the vaporizer method (EPA 2015a); the typical dose per hive using the vaporizer method is about 2 g OAD.

Other uses

OA is a reducing agent that can be used as an analytical reagent and standard, a wood bleach, a metal polish, and a metal precipitating or chelating agent (Walter 1996). It is used as a stain remover and mordant in the textile industry (Cassar and Gardano 1978; CAMEO 2016; Zaher, Fritzler and Hutchinson 2005; NCBI 2018; Vishwakarma and Gogate 2011) and as a cleaning and sterilizing agent that removes calcium in water treatment applications (Vishwakarma and Gogate 2011). It has reportedly been used to hydrolyze corncobs as a pre-treatment in biofuel production (Lee, et al. 2011) and applied as an environmental control for ticks (Valcárcel, et al. 2014). Reimenshneider and Tanifuji (2011) quantified its principal applications as approximately 28 percent metal treatment, 25 percent textile treatment, 20 percent bleaching agents, and 27 percent chemical uses. The petitioned use was not specifically addressed in these estimates but may be considered under the category of chemical uses.

Approved Legal Uses of the Substance:

In 2015, the EPA approved the registration of OA as an active pesticide ingredient for the control of varroa mites in honey bees under FIFRA Section 3(c)(5) in three approved application methods: by solution to package bees, by solution to beehives, and by vapor treatment of beehives (EPA 2015a). Currently, two companies have sub-registrations for these treatments: Chemicals Laif S.P.A., under EPA Registration Number 91266-1-73291, and Brushy Mountain Bee Farm, under Registration Number 91266-1-91832.

EPA-registered pesticide products are also regulated by individual states, including OA used in apiculture. As of November 13, 2018, the two registrants noted above are not registered by all 50 state programs (National Pesticide Information Center 2018c).

- EPA Registration Number 91266-1-73291 is not registered by the following states: California, Connecticut, Illinois, Maryland, Massachusetts, New Hampshire, South Dakota, and Texas.
- EPA Registration Number 91266-1-91832 is not registered in California.

California is the only state without a state-registered OA product for apicultural use (CDPR 2018). One product has begun the registration process but has not yet received final approval.

OA is exempt from the requirement of a tolerance at 40 CFR 180.910 when used as an inert ingredient pre- and post-harvest as a calcium chelating hard water inhibitor. The regulation requires that no more OA should be used than is necessary to chelate calcium, and in no case should more than 2 lbs OA per acre be used (EPA 2004).

The FDA permits the use of OA as an indirect additive used in food contact substances under 21 CFR 177.1010 (for use as a polymerization catalyst aid in polymers used as basic components of single and repeated use food contact surfaces) and under 21 CFR 177.2410 (as an optional catalyst in the production of phenolic resins).

Action of the Substance:

On mites

There are many studies on the efficacy of OA on *V. destructor*, but studies demonstrating the mechanism by which it acts are more limited. Experiments have thus far indicated that OA acts as a poison through direct contact with mites rather than as a systemic poison accumulating within the fluids of treated bees (Rademacher, Harz and Schneider 2017; Milani 2001; Aliano, Ellis and Siegfried 2006). Once in contact with the mites, the acidity of OA appears to be responsible for mite mortality, as opposed to some feature of oxalate ions that might form (Nanetti 1999). One study found that different parts of mite bodies appear more susceptible; mites found lying on their backs on surfaces treated with OAD were less prone to injury (Milani 2001). Al Toufailia et al. (2015) found that OA damages varroa mite mouthparts. Another experiment comparing several acids other than OA found that pH alone did not directly correspond to mite mortality (Higes, Martín-Hernández and Meana 2006).

One study evaluated the action of different OAD treatments: by sublimation (1 g OAD per hive), oral application (70 µg per bee) and topical application (70 µg per bee) to the abdomen of phoretic mites. The concentrations approximate what would typically be used in a hive (Papežíková, et al. 2017). The study found OAD crystals attached to the bodies of dead mites, even for those with brief exposure of five minutes. Only around 12 percent of mites displayed cuticular damage as observed under a dissecting microscope. Besides two fatally damaged mites—which had missing legs and a split dorsal shield—the majority that sustained cuticular damage had only mild compression of the dorsal shield, which would not be expected to affect viability. These findings support the hypothesis that OA acts via contact toxicity on varroa mites rather than the OA crystals causing structural damage. The authors speculated that OA may affect the mite's ability to hold onto the bee's body and/or its ability to re-infest the host. The group found that oral treatment of bees with OA also caused mite mortality, despite there being no direct contact of the mites with OAD crystals. The authors suggested that this could be due to metabolic disturbances and changes in the bees' hemolymph affecting the survival of the mites, which are tightly adapted to their hosts.

Mites are likely unable to detect OA by olfaction[4] (Papežíková, et al. 2017), and Ailano et al. (2006) surmised that inhalation of OA vapors is not a significant factor in the substance's toxicity to mites due to its low volatility at room temperature. However, with the sublimation method, mites would be exposed to OA in the gas phase until it crystallizes on their bodies.

On honey bees

While honey bees are more tolerant to OA than mites, they do exhibit some negative effects, especially at higher concentrations. When treated with doses insufficient to directly cause mortality (i.e., sub-lethal doses), bees experience decreased activity and changes to cuticle (exoskeleton) enzymes. Doses large enough to cause mortality cause internal tissue damage (Martín-Hernández, et al. 2007). Additional details on various effects are described here and in *Evaluation Question #5*.

- *Histopathological (tissue) effects:* Martín-Hernández et al. (2007) conducted histopathological tests using direct topical application of an OA solution (presumably OAD) at 660 µg/bee, which did not cause visible external damage to the subjects. However, it caused progressive damage to internal organs, including epithelial tissues of the ventriculus, Malpighian tubes, and rectum, which increased for at least 72 hours—the length of the experiment. The increased cellular damage over time suggests that the effect of OA continues after initial contact and, according to the authors, may cause permanent lesions. The authors also suggested that some OA may be ingested by bees during grooming (Martín-Hernández, et al. 2007). Radermacher et al. (2017) found that dermal application of OAD to honey bees is relatively well tolerated, but oral application is three times more toxic. Larvae subjected to a spray application of 2.97 percent OAD exhibited minor damage to the midgut (Gregorc, Pogacnik and Bowen 2004). Papežíková et al. (2017) found that trickling OAD solutions increased the rate of apoptosis in bees' midgut cells, suggesting cell damage, though food intake did not differ between treatment groups. In contrast to the findings of Martín-Hernández et al. (2007), Papežíková et al. did not find epithelial destruction in Malpighian tubules, possibly becaue there was less direct exposure via the trickling method in their study compared to direct application in the former.

- *Biochemical effects:* The glutathione S-transferase family of enzymes is common to most organisms, and serves to convert reactive molecules, including carboxylates, into non-reactive forms for the purpose of detoxification (Clark 1989). Nanetti et al. (1999) reported no difference in glutathione S-transferase (GST) activity between treated and untreated groups of bees, which led the authors to conclude that OA trickling at normal dose does not compromise the bee digestive system or weaken the detoxifying activity against potentially harmful substances. However, a study in Poland assessed the effects of oxalic acid treatment on bees' cuticle proteolytic enzymes (CPEs),

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[4] Olfaction is defined by *A Dictionary of Biology* as "The sense of smell or the process of detecting smells. This is achieved by receptors in olfactory organs (such as the nose) that are sensitive to air- or water-borne chemicals" (Hine and Martin 2015).

both proteases and protease inhibitors, and found that the concentration of hydrophobic proteases was significantly reduced with OA treatment, and both hydrophilic and hydrophobic CPE activity was lowered (Strachecka, et al. 2012). The proteolytic barrier has important functions in preventing fungal and other infections; thus, OA may depress the natural immunity of treated bees by disrupting the protyolitic activity of the cuticle (Strachecka, et al. 2012). Proteolytic enzymes are also present in bees' alimentary duct, hemolyph, moult liquid, and venom.

- *Sublethal effects:* Bees exhibit behavioral changes when treated via trickling at sub-lethal doses of OA. At 175 µg OAD/bee, Schneider et al. (2012) found that the treatment caused a decrease in worker activity, nursing behavior, and longevity, but caused an increase in grooming behavior (2012). Neurotoxic damage was suspected as one possible cause of the change in behavior based on similar results from another study where bees treated with formic acid were immobilized and did not regain mobility. However, the authors also acknowledged that the changes in bee behavior could have resulted from damage to the digestive and excretory organs, causing an insufficient resorption of nutrients through the epithelium of the gut and resultant weakening of the bees. For comparison, the dosage used in this study was roughly three times higher than the typical maximum dosage of 57 µg OAD/bee for a honey bee colony of 35,000 using the trickle method of application (Aliano, Ellis and Siegfried 2006).

Variables
Although the mechanism by which oxalic acid acts on both varroa mites and honey bees is still being explored, studies have elucidated various parameters that have bearing on the efficacy of OA treatment, suggesting some correlation with its mode of action. These include, but are not necessarily limited to, OA concentration, frequency, formulants, exposure time, climate, and relative humidity within the hive.

- *Concentration*: Variations in mite mortality with different concentrations of OAD suggest contact toxicity (Milani 2001). One study used a 3 percent OAD concentration at a dosage of 3 µL per bee—roughly equivalent to 5 mL per seam—with an efficacy rate of 91.3 percent. The amount of OAD applied to each bee was about 90 µg (Rademacher and Harz 2006). Efficacy increased to 98.4 percent when the concentration was increased to 4.6 percent OAD. However, higher concentrations (e.g., 6 percent OAD) did not necessarily increase mite mortality and posed a greater risk for bees. A similar volume applied to twice as many bees (3.0 and 1.5 µL, respectively) with the concentration doubled from 2.3 to 4.6 percent OAD resulted in 98 percent efficacy and 80 percent mite fall (Büchler 2000, reviewed in Rademacher and Harz 2006). Charrier et al. (2015) also noted that concentration can be varied to optimize efficacy depending on climatic region.

- *Frequency*: Repeated applications of OAD result in a higher level of bee mortality (Rademacher and Harz 2006).

- *Time dependence:* Martín-Hernández, et al. (2007) observed increased cellular damage to bees over time following OA treatment. The pathological effects observed after 48 hours were severe and irreversible, leading the authors to conclude that OA continues to act on organisms following initial contact and that OA exposure can cause permanent lesions that may be time- and dose-related.

- *Climate and season*: It is widely acknowledged that oxalic acid treatments are most effective during broodless periods because OA does not affect varroa mites in capped brood cells (Gregorc and Planinc 2001; Bacandritsos, et al. 2007). For this reason, application is recommended for late fall or early spring in temperate climates when brood are most often absent. OA efficacy is greatly reduced in warmer climates where brood rearing periods are much longer (Higes, Meana and Suárez 1999; Rademacher and Harz 2006; Higes, Martín-Hernández and Meana 2006). According to the EPA-registered label for OAD, application of OAD when brood is present might also damage the brood (EPA 2015c).

- *Formulants and relative humidity*: Sugar and, to a lesser extent, glycerol used in combination with OA appear to have a synergistic effect in increasing mite mortality. Milani (2001) demonstrated that neither sucrose or glycerol increased mortality over controls on their own, indicating that they improved dispersability or had some other physical effect on OA treatments. The study noted that with both formulants the OA absorbed water at lower relative humidity, and the resulting concentrated liquid deposits of OA were more effective than dry deposits. For more details on these formulants, see *Combinations of the Substance*.

Combinations of the Substance:

The petition for oxalic acid lists ancillary substances as "not applicable" (Domeier 2017). Specifications for impurities in OAD as a chemical reagent are: no more than 0.005 percent insoluble matter or sulfate, 0.002 percent chloride, 0.001 percent calcium or nitrogen compounds, and no more than 5 ppm heavy metals, or 2 ppm iron (American Chemical Society 2006).

The label for EPA Registration No. 91266-1 lists OAD as 97 percent of the product composition, with 3 percent inert ingredients. Information regarding the identity of the inert ingredients is not publicly available.

The EPA registered label instructs, for the solution method, to dissolve 35 g of OAD in one liter of a 1:1 sugar-to-water (weight:volume) ratio. This syrup acts as a sticker, spreader, and carrier. Proportions of sugar and water in applied OAD solutions are commonly 1:1 but can range from 0–60 percent sugar, especially in experimental applications (Mutinelli, et al. 1997). Nanetti et al. (2003) found that for a given OA concentration, 60 percent sugar solutions were more effective than 30 percent sugar solutions.

In addition to sugar, glycerol has been cited for potential use as a humectant, or moisture retention aid, for improving the application of OA solutions due to its high viscosity and good distribution. In one study carried out in Argentina, OA and glycerol were used to impregnate cellulose strips placed in hives to control mites. The authors thought that the glycerin may help maintain the OA's efficacy in the hive for a longer period (Maggi, et al. 2015). The combination of glycerol with OA may also prohibit bees' oral ingestion of OAD. It should be noted that glycerol has not been approved in the United States as a veterinary drug in combination with OAD (Rademacher, Harz and Schneider 2017).

While not combined temporally with other active miticides, the EPA recommends OA be used in rotation with other treatments for varroa control to minimize the opportunity for mites to develop OA resistance (EPA 2015a). This rotational use is discussed in *Evaluation Question #11*.

Status

Historic Use:

OA is one of the oldest known acids, having been identified as a component of plants in the eighteenth century and chemically synthesized in the first half of the nineteenth century (Riemenschneider and Tanifuji 2011).

The parasitic mite *Varroa destructor* began parasitizing the Western honey bee (*Apis mellifera*) in the 1960s (CABI 2017) after transferring from its original host, the Asian honey bee (*A. cerana*) (Adjlane, Tarek and Haddad 2016). The mite had previously been identified as *Varroa jabosoni*, but scientists distinguished the haplotypes, or genetic variants, infesting *A. mellifera* as a different species, *Varroa destructor* (Anderson and Trueman 2000). Infestations on *A. mellifera* subsequently spread globally from Asia to Europe, Africa, and the Americas (Colin, García Fernández and Ben Hamida 1999). Acaricides such as coumaphos and synthetic pyrethroids have been used to control the mites since their appearance; however, the mites developed resistance to these controls, necessitating the development of new controls (Rademacher and Harz 2006). The effective use of OA against varroa mites has been documented since the early 1980s in Japan (Nanetti, Büchler, et al. 2003).

Organic Foods Production Act, USDA Final Rule:

OA does not appear in the Organic Foods Production Act or in the USDA organic regulations, 7 CFR Part 205.

International

Canadian General Standards Board Permitted Substances List

OA is allowed for organic livestock (apiculture) healthcare under the Canadian Organic Standards as follows:

- CAN/CGSB-32.310-2015 Clause 6.6.10: *"The use of veterinary medicinal substances shall comply with the following: (a) if no alternative treatments or management practices exist, veterinary biologics, including vaccines, parasiticides or the therapeutic use of synthetic medications may be administered, provided that such medications are permitted by this standard and Table 5.3 of CAN/CGSB-32.311 or are required by law."*
- CAN/CGSB 32.311-2015 Table 5.3: Healthcare products and productions aids as follows: "*Oxalic acid: For mite control in honeybee colonies"*

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

OA is allowed for organic livestock (apiculture) healthcare under the CODEX Alimentarius guidelines per GL 32-1999, Rev. 1-2001 as follows: Annex 1: Principles of organic production, B. Livestock and Livestock Products;

- Health Care, 22: *"The use of veterinary medicinal products in organic farming shall comply with the following principles: a) where specific disease or health problems occur, or may occur, and no alternative permitted treatment or management practice exists, or, in cases required by law, vaccination of livestock, the use of parasiticides, or therapeutic use of veterinary drugs are permitted;"*
- Species Specific Requirements: Beekeeping and bee products: Health of the bees, 71: *"For pest and disease control the following are allowed: lactic, oxalic, acetic acid…"*

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

OA is allowed for organic livestock (apiculture) healthcare under the European Economic Community (EEC) per Council Regulations (EC) No 889/2008 as follows:

- EC No 889/2008: Chapter 2 (Livestock production): Section 4 (Disease prevention and veterinary treatment), Article 25 (Specific rules on disease prevention and veterinary treatment in beekeeping): *"6. Formic acid, lactic acid, acetic acid and oxalic acid as well as menthol, thymol, eucalyptol or camphor may be used in cases of infestation with Varroa destructor."*

Japan Agricultural Standard (JAS) for Organic Production

OA is not allowed for use in organic livestock healthcare, including apiculture under the JAS for Organic Production. The JAS does not include bees under the definition of livestock, nor does it include other references to apiculture.

IFOAM – Organics International

OA is allowed for organic livestock (apiculture) healthcare under the IFOAM Norms for Organic Production and Processing as follows:

- 5. Animal Husbandry: 5.8 Bee Keeping: *"5.8.7 For pest and disease control the following are permitted: …b. oxalic acid, acetic acid;"*
- Appendix 4 – Table 2: Indicative list of equipment cleansers and equipment disinfectants: *"Oxalic acid"*

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?

A) OA is a livestock parasiticide.
B) OA is a not an inert ingredient.

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)).

Although the available literature confirms that commercially available sources of OA are chemical sources, the specific manufacturing process used to manufacture the OAD ingredient used in EPA-registered varroa mite control products is unknown. Several authors (Riemenschneider and Tanifuji 2011; Betiku, Emeko and Solomon 2016; Mandal and Banerjee 2005) state that OA is usually produced through the oxidation of carbohydrates or alkenes (including ethylene or propene), or synthesis from carbon monoxide and water. Other methods may still be used by small firms, including the sodium formate method, alkali fusion of cellulose, and the isolation of oxalic acid as a byproduct of fermentation (Riemenschneider and Tanifuji 2011). Many methods share similar features: a) organic (carbon-containing) molecules are oxidized with nitric acid and catalysts to form oxalic acid; b) organic molecules are oxidized and form salts, which are then purified using sulfuric or hydrochloric acid to form OA and calcium sulfate or sodium chloride (Zaher, Fritzler and Hutchinson 2007); or c) OA is synthesized from smaller molecules like carbon dioxide or carbon monoxide.

In all these processes, OAD crystals are produced through precipitation of OAD from a mother liquor. Crystallization can be initiated by cooling OAD solutions to reduced solubility (Lidbury 1912; Young 1924), solvent evaporation or distillation (Lidbury 1912; Cassar and Gardano 1978; Vuori and Mattila 1991), supersaturation (Krochta 1989; Kharas 2014), or spray drying (Twardowski, et al. 2016). To form AOA, OAD is heated to 95-100°C (Beckham 1954; Riemenschneider and Tanifuji 2011).

Chemical sources (common methods):

- *Carbohydrate oxidation:* Carbohydrates from a variety of sources are hydrolyzed to glucose monomers by boiling in oxalic or sulfuric acid. Glucose is then reacted with nitric acid in the presence of vanadium pentoxide and iron to form OA, nitrogen oxides (which are recovered), and water (Fuchs and Watson 1970). OA may be produced this way in Spain, Brazil, China, Taiwan, Korea, and India (Riemenschneider and Tanifuji 2011).
- *Ethylene glycol oxidation:* Ethylene glycol is reacted with nitrogen tetroxide and oxygen or nitric acid and sulfuric acid to form OAD in a one-step process (Young 1924; Yonemitsu, et al. 1972). In the Mitsubishi Gas Company process, carbon dioxide is the primary byproduct, and catalysts such as vanadium pentoxide are not required (Yonemitsu, et al. 1972). According to Riemenschneider & Tanifuji (2011), most of the OA produced in Japan uses this method.
- *Propene oxidation:* Propene, generated from petrochemical refinement, is partially oxidized with nitric acid to form α-nitratolactic acid, lactic acid, nitrous oxide, and water. The α-nitratolactic acid is reacted with oxygen and catalysts (such as chromium or iron nitrate and tin chloride) to form oxalic acid, carbon dioxide, nitric acid, and water. This process can form unstable intermediates that can lead to violent explosions, and is performed by only one company, located in France (Riemenschneider and Tanifuji 2011).
- *Synthesis from carbon monoxide and water:* Carbon monoxide is reacted with alcohol (such as butanol) under pressure, using a platinum or palladium salt and ferric or cupric chloride salt as catalysts. Then, either oxygen is added, or a DC electric current is used to maintain an oxidized state, to keep the salts functioning catalytically (Fenton and Steinwand 1968). In the first step, an OA diester is

formed from the carbon monoxide and alcohol, which is then hydrolyzed to form OA and regenerate the original alcohol (Riemenschneider and Tanifuji 2011). The method can vary through additions of ammonia (Cassar and Gardano 1978).

Chemical sources (other less common methods):

- *Synthesis from carbon dioxide and water:* Kharas (2014) and Twardowski et al. (2016) developed methods whereby carbon dioxide and water are electrochemically converted to OA using electrolyzed water, created using electrochemical catalysts such as cobalt and a platinum-group, or using similar metal and a lithium salt.
- *Synthesis from sodium formate:* Carbon monoxide is absorbed into a heated solution of sodium hydroxide under pressure to form sodium formate, which is then evaporated and heated again to form sodium oxalate, sodium carbonate, and hydrogen gas. The sodium oxalate is treated with calcium hydroxide and water to precipitate calcium oxalate and simultaneous regeneration of sodium hydroxide. The calcium oxalate is treated with sulfuric acid to form OA and calcium sulfate (Wallace 1926). Alternatively, sodium oxalate can be treated with nitric acid to form OA and sodium nitrate (Beckham 1954). Riemenschneider & Tanifuji (2011) note that some of the steps involved with the sodium formate method are cumbersome and are of questionable safety but may still be used in China.
- *Alkali fusion of cellulose:* Cellulosic material such as sawdust is reacted with alkali such as sodium hydroxide under pressure, which forms sodium oxalate (Lidbury 1912; Bannister 1934; Krochta 1989). Bannister (1934) speculated that the cellulosic material first decomposed into formic acid, and then formic acid decomposed further to OA. Sodium oxalate is converted to OA through treatments with sulfuric acid, similar to that used in the sodium formate method (Riemenschneider and Tanifuji 2011). The Merck Index (O'Neil, et al. 2013) notes that the alkali fusion method has been replaced by other methods.

Natural sources:

- *Microbial fermentation:* As reported by Sauer et al. in 2008, existing market data did not show that microbial fermentation sources were used for OA. In contrast, Riemenschneider and Tanifuji (2011) stated that OA may be produced in some parts of the world as a fermentation byproduct during the manufacture of other substances such as citric acid, but the process required large amounts of water and was not economically feasible at that time. Since then, Kobayashi et al. (2014) genetically transformed a strain of *Aspergillus niger* which overexpressed a single enzyme—oxaloacetate hydrolase (encoded by the oahA gene)—and hyperproduced OA in liquid media. This strain produced a yield of 96.3 percent OA based on the glucose consumed. In a report from 2016, a strain of *A. niger* was developed through artificial selection, which produced 123 g of OA/kg dry weight of corncob using a solid-state fermentation process (Mai, Lee and Choi 2016). There is additional interest in further development of OA production through microbial fermentation (Nakata and He 2010; Emeko, Olugbogi and Betiku 2015; Mai, Lee and Choi 2016; Betiku, Emeko and Solomon 2016).

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)).

Based on available literature, all commercially available OA is produced through chemical processes (Riemenschneider and Tanifuji 2011; Betiku, Emeko and Solomon 2016; Mandal and Banerjee 2005), including the oxidation of larger molecules using acids, or the synthesis of smaller molecules using pressure and catalysts. These processes are detailed above in *Evaluation Question #2*.

Riemenschneider and Tanifuji (2011) speculated that some developing nations might use fermentation to produce OA, but the market data summarized by Sauer et al. (2008) did not show such processes are used. Although there is interest in producing OA through microbial fermentation, and yield is improving dramatically in some cases (Nakata and He 2010; Emeko, Olugbogi and Betiku 2015; Mai, Lee and Choi 2016; Betiku, Emeko and Solomon 2016), no sources positively identified fermentation as a current commercial production method.

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)).

Naturally occurring OA is ubiquitous in the environment. It has low persistence and no potential for bioaccumulation in the food chain (Chem One Ltd. 2015). OA released into the soil under environmental conditions (ie., pH 5–9) is found in the oxalate ion form and readily biodegrades (U.S. National Library of Medicine 2005; Environment Directorate General of the European Commission 2014). OA begins biodegradation under anaerobic and aerobic conditions in less than one day (EPA 1992). One Material Safety Data Sheet for commercial OA states aerobic biodegradability as being 89 percent after 20 days exposure time and classifies this as being readily biodegradable (Penta International Corp. 2018). Aerobic and anaerobic biodegradation are the predominant fate of OA in subsurface waters. OA does not volatilize at room temperature, adsorb to sediment, bio-concentrate in aquatic organisms, or oxidize or hydrolyze in water under natural conditions. In surface waters and on soil surfaces, OA primarily undergoes photolysis (breakdown by light). Daytime persistence of OA on soil surfaces is expected to be less than a few hours. In the atmosphere, the amount of OA that can be supported by cloud water is 9–18 ng m^3 for short-lived clouds, and 28–56 ng m^3 for long-lived clouds where the chemistry stabilizes (Warneck 2003). The atmospheric fate of OA is likely to be wet or dry deposition, followed by biodegradation as described. OA may also undergo photolysis in the atmosphere (U.S. National Library of Medicine 2005).

Degradation methods and by-products

In all its degradation pathways, OA primarily decomposes into carbon dioxide and water, and to a lesser extent carbon monoxide and water. As a reducing agent, OA reacts readily with oxygen, yielding carbon dioxide and water (Riemenschneider and Tanifuji 2011). Under normal circumstances, the decomposition temperature of OAD is 157°C (LabChem 2018), at which point it decomposes into carbon monoxide, carbon dioxide, and water with formic acid as an insoluble intermediate (Riemenschneider and Tanifuji 2011). The decay of OA to carbon dioxide may also be biologically or enzymatically mediated, as happens to OA in nature with white rot and brown rot fungi depolymerizing wood hemicellulose (Espejo and Agosin 1991). AlSalka, et al. (2018) reported that in aqueous solutions, gamma- or X-rays induce decomposition of OA to carbon dioxide and hydrogen at a molar ratio of 2:1 and generate carbon monoxide and formic acid. The authors observed complete photocatalytic degradation of OA into carbon dioxide and hydrogen within 60 minutes of illumination under aerobic conditions with no byproduct detected and with only minor formation of formic acid under anaerobic conditions (AlSalka, et al. 2018). OA is convertible to formic acid and vice versa (Walter 1996).

Residues of OA in honey

Although OA occurs naturally as a minor component of honey, several studies have found that treating beehives with OA does not leave toxicologically significant residues in honey. Mutinelli et al. (1997) found that the difference in OA content of honey taken from the nest of hives pre- and post-OA treatment was not significant and concluded that treating bees for varroa mites with OA can be done without causing an increase in the OA concentration in the honey. Maggi et al. (2015) reported that there was no increase in OA content of honey, beeswax, or bees after treatment with OA strips. Rademacher and Harz (2006) attributed the lack of accumulation of OA residues in beeswax and propolis to the hydrophilic properties of OA. Charrière and Imdorf (2002) reported no risk of OA residues in honey resulting from repeated treatment of hives via trickling or spraying of OA. Rademacher and Harz (2006) reported that increased OAD levels in honey following repeated OAD applications have been observed in amounts of 76.3 mg/kg from trickling and 62.8 mg/kg from spraying, but these elevated levels did not exceed those seen from natural variation in the OA content of honey from different botanical sources. Health Canada's Pest Management Regulatory Agency (PMRA) also concluded that the amount of OA present as a residue after application for varroa control in hives is not expected to exceed the range of naturally occurring concentrations found in honey (PMRA 2010).

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

The mode of action of OA on both varroa mites and honey bees is discussed above under *Action of the Substance*. The persistence and areas of concentration of OA and its degradation byproducts are discussed above in *Evaluation Question* #4. OA is classified as a hazard class 1, or low danger, for water and has low toxicity for fish and bacteria (Riemenschneider and Tanifuji 2011). It has been reported to inhibit the activity of some bacteria during natural interactions between fungi and plants (Nagarajkumar, et al. 2005).

Toxicity of OA to honey bees

Multiple studies found varying degrees of toxicity to bees, and researchers and applicators have sought to maximize mite fall while minimizing toxic effects on bees by adjusting concentration, dosage, frequency, and timing of OA applications. While some studies reported no detectible adverse effects of OA application on bees (Mutinelli, et al. 1997; Gregorc and Planinc 2001; Al Toufailia, Scandian and Ratnieks 2015; Gregorc, Alburaki, et al. 2017), others report varying degrees of deleterious impacts including queen loss, increased mortality, decreased brood, weakened brood, and colony failure (Higes, Meana and Suárez 1999; Higes, Martín-Hernández and Meana 2006, Martín-Hernández, et al. 2007; Adjlane, Tarek and Haddad 2016).

Papežíková et al. (2017) examined the effect of OAD treatment on bees' lifespan, finding that lifespan had not significantly decreased 21 days after OAD treatment by sublimation, but that it had significantly decreased with the trickling method. Another study examining longer-term effects found that OA treatment of beehives resulted in decreased numbers of brood and some queen death during the spring following autumn application, a time lapse of 3–4 months (Higes, Meana and Suárez 1999). Papežíková et al. (2017) noted the possibility of delayed toxic effects of OA treatment on bees, and suggested these be further examined in overwintering colonies. Some studies report a loss of about 1,000–2,000 bees, or about 6 percent of a typical overwintering colony in temperate field conditions (Charrierre and Imdorof 2002).

The lethal dose of OAD to 50 percent of a bee population after 72 hours, or the 72-hr LD_{50}, was assessed in several studies. Martín-Hernandez et al. (2007) found the 72-hr LD_{50} to be 530 µg per bee. Another study estimated the 72-h LD_{50} for bees to be lower, at 195 µg OAD per bee (Aliano, Ellis and Siegfried 2006). In the latter study, doses of less than 100 µg OAD per bee did not cause significant mortality after 48 hours (48-h LD_{10} 176.68 µg OAD per bee). Significant mortality occurred only after 72 hours following treatment. The EPA-registered label for OAD specifies a dosage of 35 g OAD dissolved in 1 liter of solution and applied at a rate of 50 mL per colony. Assuming 35,000 bees per colony, 0.05 mg or 50 µg of OAD is applied per bee on average, which is below the reported 72-hr LD_{50} levels. Rather, the applied dosage is about one-third the chronic toxicity of 175 µg OAD/bee reported by Schneider et al. (2012) and about one-fourth the LD_{50} reported by Aliano et al. (2006). However, Aliano applied doses of OAD to individual bees in acetone, and the acetone may have contributed to the toxicity.

Toxicity of degradation by-products

None of the literature reviewed for this report suggested concern over environmental concentrations of carbon dioxide and carbon monoxide resulting from the degradation of OA used in the treatment of honey bee hives for varroa mites. Although carbon dioxide and carbon monoxide are considered greenhouse gases, the application rates of OA used in beehives are miniscule compared to industrial sources of these pollutants.

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

The methods for manufacturing OA involve numerous chemicals (see *Evaluation Question* #2). In the carbohydrate oxidation process, vanadium pentoxide—a naturally occurring metal compound—is used as a catalyst. Vanadium pentoxide is considered an air pollutant, predominantly created from oil combustion (WHO 2001). Its use as a catalyst may present health risks in industrial settings. Inhalation of vanadium dust can cause lung irritation and adverse respiratory effects; OSHA limits exposure to 0.05 mg/m^3 as an 8 hour total weight average (NIOSH 2011). It is not clear whether environmental exposure results from the use of vanadium pentoxide in the manufacturing of OA. Nitric oxides, co-products of OA, are recovered in the process.

The ethylene glycol method produces carbon dioxide as a byproduct. In the synthesis of OA from carbon monoxide and water, butanol is used and recovered in the process. Other chemicals including metals, salts acids, and bases are used in the various processes. Despite this, no information was found in the literature specifically identifying potential environmental contamination hazards from the manufacturing of the substance.

The use of OA in honey bee hives has been cited for its low potential for environmental contamination due to OA's high degradability and the application being limited to within the hive. Charrière and Imdorf (2002) state that there are no environmental residues expected from the repeated application of OAD in hives by the various methods described, including spraying, trickling, and sublimation. While sublimation can produce low levels of OA in the air around hives following application, it is expected that these residues degrade in the environment via the same pathways described in *Evaluation Question #4*.

Despite its low potential for environmental contamination, OA is a strong acid; Riemenschneider and Tanifuji (2011), therefore, recommended that it should not be disposed of directly into the environment but should first be treated either by incineration or heating with sulfuric acid to convert it to carbon monoxide, carbon dioxide, and water. Small amounts of OA may be treated with potassium permanganate to yield carbon dioxide or neutralized with lime to produce calcium oxide, which is safe for disposal (Riemenschneider and Tanifuji 2011).

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)).

Direct chemical interactions in apicultural systems

When used as a treatment for varroa mites in honey bee hives, the use of OA is not expected to produce any reaction with other substances used in organic bee operations. None of the literature reviewed for this report suggests a reaction between OA and the sugar used as a formulant, nor does the literature recommend its use concomitant with other mite control substances. Due to its rapid degradability, no chemical interaction is expected with other treatments.

Indirect interactions and interactions beyond apicultural systems

OA is naturally occurring and as such reacts with other substances in the environment, in some cases providing important environmental functions. For example, OA reacts with calcium to form insoluble calcium oxalate, which regulates calcium concentrations in plant cells. Oxalates also provide plants natural defense to insect pests and grazers as well as tolerance to aluminum toxicity for plants growing in acidic soils. Oxalates also play a role in the phytoremediation of soils contaminated with heavy metals (Prasad and Shivay 2017). In water, its negative ion forms complexes with several different metal ions and is thereby immobilized.

OA plays a role in the pathogenicity of various fungi. In this ecological context, fungal exudates of OA precipitate calcium from the middle lamellae of plants and makes them more susceptible to fungal attack. In nature, calcium oxalate can have a depressive effect on the antibiotic activity of beneficial soil microorganisms such as *Pseudomonas fluorescens* (Nagarajkumar, et al. 2005), a beneficial bacteria used in pest control applications in organic crop production (OMRI 2018). At the same time, some strains of *P. fluorescens* can detoxify or catabolize OA.

In addition to forming both neutral and acid salts, OA reacts with most organic and inorganic bases to form crystallizing complexes, including potassium hydrogen oxalate, ammonium oxalate, and ammonium iron oxalate. OA is incompatible with strong alkalines, strong oxidizers, chlorites and hypochlorites, and combustible materials. It may react rapidly in contact with iron and iron compounds to form ferric oxalate. Contact with silver may form silver oxalate. OAD in solution is corrosive to metals (Chem One Ltd. 2015).

<u>Evaluation Question #8:</u> 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)).

Possibility of varroa mites to develop OA resistance

The potential for varroa mites to develop resistance to OA has been raised as a concern by bee keepers and the scientific community (Milani 2001); however, resistant populations have not been documented within academic literature at the time of this report. The scientific literature reports extensively on the development of varroa mite resistance to the conventional chemical controls coumaphos, pyrethroids (tau-fluvalinate), and amitraz. The former two acaricides are lipophilic substances that are absorbed in beeswax (Fries, Wallner and Rosenkranz 1998) and therefore persist and accumulate in the hive over repeated applications (Rosenkranz, Aumeier and Ziegelmann 2010; Medici, et al. 2015). It has been suggested that coumaphos residues could contribute to the development of mite resistance through the exposure of mites to sublethal doses inside brood cells (Medici, et al. 2015).

There is disagreement in the scientific literature about whether OA accumulates in beeswax. While conventional acaricides persist in wax within the hives, some authors report that organic acids such as OA and essential oils do not persist or build up in the wax (Maggi, et al. 2017; Imdorf and Bogdanov 1999) and thus are comparatively less likely to exert sub-lethal selection pressure on mites, decreasing the opportunity for them to develop resistance. However, Rademacher et al. (2017) found long-term toxic effects such as reduced brood four months following application and reported that OAD can persist in a colony for six months. In addition, radioactive OA experiments show that small amounts of OA do accumulate in the wax (Nanetti, Bartolomei, et al. 2002).

High frequency and extended duration of use are other factors that can affect the development of resistance to a substance. Milani (2001) suggested that the prolonged used of OA to treat varroosis could contribute to the emergence of resistance, as even a small increase in mites’ tolerance would greatly limit its efficacy because increasing concentrations of OA would be toxic to bees. Milani noted that the high efficacy of OA in the absence of capped brood, its ease of use, and low cost could lead to an increase in OA use and to an accompanying increase in selective pressure on mites.

Maggi et al. (2017) carried out research to assess the potential development of resistance by varroa mites to OA. Their study examined the susceptibility of mite populations that had been subjected to 64 consecutive OA treatments over eight years compared to populations that had never been exposed to OA. They found that the mites that had received ongoing OA treatment remained susceptible to OA, which demonstrated that the varroa mites did not develop resistance to OA despite repeated exposure. Several other researchers have surmised low probability for mites developing resistance to OA (A. Gregorc 2005; Papežíková, et al. 2017), stating that the selection pressure should be low due to OA’s rapid degradation inside the colony and infrequency of application, especially as compared to synthetic acaricides. At the time of the report by Maggi et al. (2017), no cases of OA resistance had been documented in varroa mites. Nevertheless, the authors did not rule out the potential for resistance development in the future and recommended continued use of precautionary measures to prevent it.

A different mechanism by which mite populations could develop resistance to OA is through the microflora associated with the mites and/or bees. Maddaloni and Pascual (2015) isolated from *Varroa destructor* certain bacteria that possess a rare trait known as oxalotrophy, or the ability to degrade OA by using it as their sole carbon source. If the association with these bacteria helps varroa mites survive OA treatment, that association could expand or increase through selective pressure, thereby leading to lower susceptibility of mite populations to OA treatment in the future (Maddaloni and Pascual 2015). The literature review for this report did not identify any documented cases of this phenomenon to date.

<u>Evaluation Question #9:</u> 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)).

OA breaks down under environmental conditions relatively rapidly, as discussed in *Evaluation Question #4*. Potential for spillage or leakage during application exists; however, given its natural occurrence in the environment and its likelihood to rapidly transform into relatively benign byproducts, OA is not expected to pose a significant risk to the environment (PMRA 2010). Its potential harm to bees is discussed in more detail under *Action of the Substance* and elsewhere in this report.

<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)).

OA is acutely toxic to humans when ingested, inhaled, or when it comes into contact with the body. It absorbs relatively well through the skin and is easily absorbed through mucous membranes, which can lead to absorptive poisoning (Riemenschneider and Tanifuji 2011). OA is also corrosive to the skin (ILO and WHO 2009). Chronic skin exposure causes localized pain and may lead to dermatitis, slow-healing ulcers, and gangrene. The oral minimum lethal dose in humans (LD_{Lo})—the lowest amount of OA required to induce death—has been reported as 4–5 g, and elsewhere as 71 mg per kilogram (kg) of body weight (which is equivalent to 4.26 g for a 60-kg person) (Riemenschneider and Tanifuji 2011). The amount of OA applied to each beehive via the vaporizer method, 2 g OAD, is 40 percent of the minimum lethal dose. OA dust or vapors cause irritation or severe burning of the eyes and upper respiratory tract. Signs of chronic exposure via inhalation include chronic coughing, vomiting, and general weakness (Riemenschneider and Tanifuji 2011). OSHA standards limit the permissible exposure to OA to a time weighted average of 1 mg/m^3 over 8 hours (NCBI 2018).

OA, while acutely toxic, does not pose risk for any systemic effects within the body (Rademacher and Harz 2006). However, in one study, anhydrous OA was tested for biological activity in mice; at a concentration of 0.2 percent AOA in drinking water, it was found to reduce water consumption to the extent that the mice had fewer litters per pair, lower pup weight, and decreased prostrate weight in the absence of detected somatic organ changes. Secondary effects of the treatment in the next generation mice were increased kidney weight, fewer live pups per litter, and increased abnormal sperm forms (Lamb, et al. 1997). A study in rats tested the oral median lethal dose (LD_{50}) of OA—the dose required to kill half the members of the tested rat population after a specified test duration. The LD_{50} for rats was reported as 475 mg per kg of body weight for males and 375 mg per kg of body weight for females (EMEA 2003).

Notwithstanding its acute toxicity at higher concentrations, OA is a natural minor component of many foods, including plant-based foods and honey. Because OA is naturally occurring in food and is not a food additive, there is not an established Allowed Daily Intake (ADI); however, one study suggested an ADI of 0.89 mg/kg, corresponding to an intake of 53.4 mg/day for a 60 kg human (Rademacher and Harz 2006). As discussed under Evaluation Question #4, the amount of OA present in honey as a residue after application for varroa treatment in hives is negligible, approximately 60–80 mg/kg (EPA 2015a), and is not expected to exceed the range of naturally occurring OA concentrations found in honey (PMRA 2010). The EPA has reported this range as being 1–800 mg/kg. In comparison, plants are reported to contain from 5 mg/kg to 200 g/kg (EPA 2015a; EMEA 2003). The estimated daily dietary intake of OA is estimated to be 50–500 mg/day, or 0.8–8.3 mg/kg/day for a 60 kg person, with wide variation based on types of food consumed (EMEA 2003). This exceeds the suggested ADI of 0.89 mg/kg, though most dietary OA is in the form of salts, which dissolve in the stomach, precipitate again in the colon, and are excreted. Overall, the use of OAD as petitioned is not expected to result in dietary risk from food consumption (PMRA 2010; EPA 2015a).

Health Canada's Pest Management Regulatory Agency (PMRA) evaluated OA for use as a control agent for varroa mites in honey bee hives, and its registration in Canada was approved in 2010. Subsequently, the EPA was able to expedite the registration of OA in the United States due in part to a NAFTA "work share" agreement in which PMRA officials provided data reviews to EPA risk assessors and risk managers (EPA 2015a). These registration decisions were based on the assessment of "reasonable certainty that no harm to human health, future generations, or the environment will result from use or exposure to the product under its conditions of registration." The assessment considered unique characteristics of sensitive subpopulations, such as children, as well as organisms in the environment most sensitive to contamination. The agency's conditions for registration of the substance included precautionary measures on the label to reduce risk (PMRA 2010).

PMRA (2010) acknowledged that exposure to OAD may occur when handling or applying the product in honey bee production. However, they determined that application at the doses specified would keep the level of potential exposure within that considered safe, having no adverse health effects. A study by Gumpp (2004) provided evidence that OAD, when applied correctly, poses no inhalation risk to the beekeepers' health. Concentrations measured for OA in the air after application by spraying and evaporation were lower for free-standing hives than in bee houses, but both remained below 0.3 mg/m^3, which is less than the existing exposure limit for oxalic acid of 1 mg/m^3. Rademacher and Harz (2006) also reported that OA concentration in the air surrounding apiaries where multiple systems are used may increase beyond the levels reported above. The authors recommend that colonies not be inspected directly after treatment because data are not available regarding OA concentration in the air of the hive over time. The EPA-approved label for OAD instructs handlers to use personal protective equipment (PPE) and to avoid breathing dust or fumes as well as to avoid contact with eyes, skin, and clothing. It also instructs users to wash hands and PPE directly following use (EPA 2015c).

Thus, while OA can be safely applied without compromising the health of the applicator, proper safety measures must be followed to avoid adverse health effects. This is particularly true when preparing solutions from concentrated acid, and for applicators using the vaporizer method, which requires the use of respirator, goggles, and gloves.

<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)).

A central challenge of controlling mites on honey bees is that both organisms may be susceptible to the same substances due to biological similarities (Pettis, Rose and Chaimanee 2017). Effective controls need to be more toxic to varroa mites than honey bees so that dosages can be tuned for therapeutic effect. Further complicating matters, the effectiveness of many substances (including OA) is variable, depending on climatic, seasonal, and other factors. For example, Higes et al. (1999) reported OA efficacy against mites as being 73 percent in spring and 94 percent in autumn. Gregorc and Planinc (2001) reported low efficacy of only 44 percent for OA trickled on brood, but 99 percent efficacy for OA trickled on broodless colonies. In general, OA has been found to have high efficacy, commonly between 75–99 percent, when applied during broodless periods (Nanetti 1999; Charrière and Imdorf 2002; Gregorc and Poklukar 2003; Higes, Martín-Hernández and Meana 2006; Bacandritsos, et al. 2007; Martín-Hernández, et al. 2007; Al Toufailia, Scandian and Ratnieks 2015; Adjlane, Tarek and Haddad 2016; Gregorc, Alburaki, et al. 2017).

In addition to OA, nine active ingredients have been approved by the EPA for use against varroa mites (National Pesticide Information Center 2018b). Several of these may be permitted as nonsynthetic alternatives. Two of these substances are synthetic and permitted for use in organic apiculture in accordance with 205.603(b).

The brand-name products identified below could potentially be allowed in organic livestock production because they contain an active ingredient that may comply with the NOP National List. However, these products have not been confirmed to meet all other applicable NOP requirements for use as inputs, such as verifying that nonsynthetic ingredients are confirmed as nonsynthetic under NOP Guidance 5033, and that inert ingredients comply with 7 CFR 205.603(e). None of the brand-name products identified below are currently listed as allowed inputs by OMRI or WSDA for use in organic livestock production.

<u>Nonsynthetic alternatives that are EPA-Registered or FIFRA exempt:</u>

- *Thymol and thyme extracts:* Thymol can be produced from both nonsynthetic (e.g., thyme oil) and synthetic sources (O'Neil, et al. 2013). Like OA and formic acid, thymol is a natural constituent of honey. It can be tasted in honey at a fairly low threshold of around 1.1–1.3 mg/kg in honey but dissipates within 3–4 weeks after treatment (Bogdanov, et al. 1999, Bonheví, Coll and Martínez 2016). Thymol has been used experimentally for varroa mite control with good success (Bonheví, Coll and Martínez 2016; Imdorf and Bogdanov 1999), outperforming OA in one experiment in Turkey (Emsen, Dodoloğlu and Genç 2010). Rahimi et al. (2017) found that an extract of thyme using ethanol as the solvent was effective in controlling varroa mites and had no adverse effect on honey bees. Thymol may modulate gamma-aminobutyric acid (GABA), a neurotransmitter involved with muscle activation and glandular stimulation (Rahimi, Del and Moradpour 2017).

While thymol is not exempt from EPA registration under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), thyme and thyme oil are (EPA 2015b). There are two EPA-registered products containing thymol (synthetic/nonsynthetic status unknown):

  - Apiguard (EPA Reg. No. 79671-1), produced by Vita (Europe) Limited, C/O Landis International, P.O. Box 5126, Valdosta, Ga; and
  - ApiLife VAR (EPA Reg. No. 73291-1), produced by Chemicals Laif S.P.A. (Arysta Lifescience America Inc.), Via Dell 'Artigianato, 13, 35010 Vigonza (PD), Italy.

- *Menthol:* Like thymol, menthol can be produced from either nonsynthetic sources (such as peppermint or other mint oils) or synthetic sources (O'Neil, et al. 2013). No literature was found directly discussing menthol's efficacy or mode of action against varroa mites, though it is used in the EPA registered formulation (below) in combination with other substances due to its efficacy against tracheal mites (Imdorf and Bogdanov 1999; Maeda and Sakamoto 2016).

  Menthol is not exempt from EPA registration, but peppermint oil is (EPA 2015b). One EPA registered product contains menthol as an active ingredient (in addition to thymol and oil of eucalyptus):

  - Api Life Var (EPA Reg. No. 73291-1), produced by Chemicals Laif S.P.A. (Arysta Lifescience America Inc.), Via Dell 'Artigianato, 13, 35010 Vigonza (PD), Italy.

- *Oil of Eucalyptus:* In a laboratory study, Ghasemi et al. (2011) found that fumigating with an oil extract from *Eucalyptus camaldulensis* was effective at killing 50 percent of mites at a concentration of 1.74 µL/L of air. The same material also caused mortality to 50 percent of bees at a concentration of 3.05 µL/L air.

  Api Life Var, the EPA-registered product mentioned under menthol, contains oil of eucalyptus as an active ingredient in addition to thymol and menthol.

- *Hop beta acids/lupulones (HBA):* HBA both repels and causes mortality in mites (DeGrandi-Hoffman, et al. 2012). In both laboratory and field studies, HBA increased mite mortality over controls and was found to be more effective than the synthetic acaricide, fluvalinate. HBA in colonies is ephemeral, disappearing in less than 2 weeks; therefore, multiple treatments are needed to effectively control mites. When treating packaged bees in 0.9 kg containers with strips impregnated with HBA, over 90 percent of mites were killed, with no statistically significant increase in honey bee mortality (DeGrandi-Hoffman, et al. 2012). Larger packages treated with 4 strips had elevated bee mortality compared to controls (2.7 ± 0.4% for treatment, compared with 0.62 ± 0.12% for control).

  One EPA-registered product contains hop beta acid resin:

  - Hopguard II (EPA Reg No. 83623-2), produced by Betatec Hop Products, a subsidiary of John I. Haas, Inc., 5185 Macarthur Blvd., NW – Suite 300, Washington DC, 20016.

<u>Nonsynthetic alternatives that are not EPA registered or FIFRA exempt:</u>

- *Other essential oils (other than thymol, menthol, and oil of eucalyptus):* Also known as "ethereal oils," essential oils can either attract or repel varroa mites (Kraus, Koeniger and Fuchs 1994). Oils that attract mites included clove, cinnamon, and menthone. Though not attractive enough to be used as a bait, cinnamon oil causes increased mite mortality. In a laboratory experiment, citronella oil was the most repellent oil, but oil of marjoram was more effective in the field. When embedded in comb foundation wax, oil of marjoram decreased the infestation rate of varroa mites by 46–48 percent. The toxicity of many essential oils is only slightly different for mites and bees, making them unsuitable for use to directly kill mites (Kraus, Koeniger and Fuchs 1994).

Imdorf and Bogdanov (1999) noted that other than thyme, only a few essential oils have been used successfully as acaricides in field applications: wintergreen oil in combination with a thermal treatment; aerosol spray of thyme-sage oil mixture; and passive evaporation of thymol, oregano oil, and marjoram oil in combination with diluted formic acid. Other essential oils toxic to mites, but not to honey bees at similar concentrations, include lavender, lavendin, and laurel oils (Damiani, et al. 2009).

Numerous essential oils are FIFRA-exempt as active ingredients, including cinnamon oil, clove oil, and peppermint oil. However, the more efficacious oils noted above (except for thyme oil) are not exempt when used as an active ingredient in a pesticide.

- *Neem oil:* One study demonstrated that when sprayed six times at four-day intervals, neem oil killed 50–80 percent of mites, but also reduced the honey bee brood population by 50 percent and caused up to 50 percent losses of honey bee queens (Melathopoulos, et al. 2000). Neem seed extracts can disrupt hormones related to honey bee caste determination (i.e., what role the honey bee will play within the colony) and cause larval deformities and death within 48 hours of application (Rembold, et al. 1980). Neem oil is a contact insecticide composed of numerous chemical compounds including azadirachtin. While little is known about its mode of action, azadirachtin may stimulate cells involved with feeding inhibition, weakening, and killing insects (Campos, et al. 2016).

  There are currently no EPA-registered neem oil formulations for use on honey bees to control varroa mites (National Pesticide Information Center 2018b), and neem oil is not noted as a FIFRA-exempt active ingredient in pesticide formulations (EPA 2015b).

- *Rotenone:* Rotenone is a nonsynthetic botanical pesticide (isoflavonoid) made from plants in the Fabaceae family (legumes), such as *Derris* spp., *Lonchocarpus* spp., and *Tephrosia* spp. It is acutely toxic to insects and fish species, blocking the process of cellular respiration within mitochondria (EPA 2007). In one study, rotenone caused an average mite mortality of 27 percent, which was as effective as OA (with an average mite mortality of 20.85 percent) for controlling varroa mites when brood was present (Gregorc and Poklukar 2003). Its effect on bees was not reported.

  Rotenone is currently only registered with the EPA as a piscicide, or fish pesticide (National Pesticide Information Center 2018a).

- *Nonsynthetic organic acids (acetic, citric, costic, and lactic):* Nonsynthetic organic acids such as acetic, citric, and costic acid have been compared against OA for efficacy against varroa mites. While citric acid is often produced through fermentation (Verhoff 1985), modern methods of acetic acid production are typically synthetic except for fermentation processes used for table vinegar (Aguiló, Hobbs and Zey 1985). Higes et al. (2006) found that acetic acid and citric acid were no more effective for mites than control groups but increased bee mortality. Sofou et al. (2017) found in field tests that costic acid extracted from the plant *Dittichia viscosa* was either as effective, or 80 percent as effective, as OA (depending on the field) and did not cause bee mortality.

  Acetic, citric, and costic acid are not noted as FIFRA-exempt active ingredients in pesticide formulations (EPA 2015b).

Synthetic alternatives that are EPA Registered and on NOP National list:

- *Formic acid (CAS# 64-18-6):* Formic acid is listed on the National List at 7 CFR 205.603(b)(2) with the following annotation: "for use as a pesticide solely within honeybee hives." Like OA and thymol, it is a natural constituent of honey (Bogdanov, et al. 1999). While formic acid occurs naturally, commercial forms are from synthetic sources (Hietala, et al. 2016). Unlike OA, formic acid is effective against both phoretic and reproductive mites within sealed brood cells (Rosenkranz, Aumeier and Ziegelmann 2010), but its effectiveness is variable and dependent on ambient temperature, and under some conditions (such as when formic acid evaporates too rapidly from application pads) it can harm bees (Elzen, Westervelt and Lucas 2004). In Florida, Elzen et al. found that formic acid applied on a saturated pad reduced varroa mites by 39.7 percent. In Argentina, Eguaras et al. (2001) found that formic acid in a gel matrix reduced varroa mites by 92 percent.

  There are three EPA-registered products containing formic acid:
    - For-Mite (EPA Reg No. 61671-3), produced by Mann Lake Ltd. (Landis International, Inc.), 501 S. 1st Street, Hackensack, Mn 56452-2001
    - Mite Away Quick Strips (EPA Reg No. 75710-2), NOD Apiary Products USA Inc., 8345 NW 66th Street #8418, Miami, Fl
    - Formic Pro (EPA Reg No. 75710-3)

- *Sucrose octanoate esters (CAS#s 42922-74-7; and 58064-47-4):* Sucrose octanoate is listed on the National List at 7 CFR 205.603(b)(8) and 205.601(e)(10)[5] with the following annotation: "in accordance with approved labelling." Originally discovered in wild tobacco, sucrose octanoate is damaging to soft-bodied insects and mites in a similar manner as insecticidal soaps (i.e., it causes suffocation or disruption of cuticular waxes) (Isman 2006). Against varroa mites, Sammataro et al. (2008) found that applying a spray of sucrose octanoate esters was no more effective than a non-treatment control.

  No active EPA registrations exist; however, the following product containing sucrose octanoate was formerly registered:
    - Avachem Sucrose Octanoate [40.0%] (EPA Reg No. 75197-1), produced by Applied Power Concepts, Inc., 411 E. Julianna Street, Anaheim, Ca 92801-2515.

OA used in rotation with other products

The scientific literature does not generally provide prescriptive instructions for an overall varroa mite management plan. The effectiveness of chemical and biotechnical controls is variable (as an example, see *Action of the Substance: Variables* for the variability in effectiveness for just OA). Governmental, academic, and beekeeper interest groups provide pest management manuals that describe general methods and chemicals for mite control, but do not typically provide specific rotation instructions with efficacy data supporting or comparing different management plans.

A variety of sources recommend using multiple measures (both chemical and biotechnical) to control varroa mites on honey bees, including the use of miticides in rotation (Charrière and Imdorf 1999; ATTTA 2017; Ritter 1999; Kristiansen 1999; Rosenkranz 1999; Caron 2015; Mullen 2016; DEFRA 2017). Using treatments with different modes of action can help prevent mite resistance and increase treatment efficacy (Rosenkranz, Aumeier and Ziegelmann 2010). None of the literature reviewed suggested OA treatments be used exclusively, and sources repeated similar, though not identical, overall management schemes.

OA treatments are best suited to broodless conditions (see *Action of the Substance*). If chemical and biotechnical control strategies are used, different ones are needed throughout all honey bee/colony life stages. Therefore, if chemical control is part of the varroa management plan, multiple chemicals may be beneficial. Ritter (1999) divided varroa control into three phases:

1) application of biotechnical (non-chemical) controls at the beginning of the season;

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[5] The reference to sucrose octanoate esters at 7 CFR 205.601(e)(10) is specific to insecticidal and acaricidal (for mite control) uses in crop production, and does not apply to apicultural uses under the Livestock scope.

2) monitoring and chemical treatments when necessary during the summer; and

3) verification of treatment success and application of additional controls as necessary (of all types) in the late season.

Similar to Ritter, Kristiansen (1999) recommends drone brood removal in spring (where reproductive mites concentrate), followed by evaluation of mites in June, application of formic acid after honey harvest, and finally applying lactic acid or oxalic acid as necessary. Charrière and Imdorf (1999) stress the importance of mite reduction in August and September using formic acid or thymol, followed by OA (or coupmaphos) in November. According to Ritter, the complete treatment concept applied is more important than the specific chemicals used.

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

Five general approaches are used for non-chemical control of varroa mites: developing resistant honey bee varieties, using screens at the bottom of colonies to trap and sequester mites away from honey bees, dusting bees to knock down the population of phoretic mites, removing brood, and heat treatments. These methods are often complicated, time-consuming, and dependent on proper timing (DEFRA 2017) and may not be suitable for all operations.

Resistant/tolerant varieties

The varroa mite's natural host is the Asian hive bee (*Apis cerana*). This bee is both mite tolerant (i.e., bees reduce the harmful effects of the mites) and mite resistant (i.e., bees reduce the reproductive success of the mites). The bees control mites by killing phoretic individuals and removing mite-infested bee pupae from the colony. Mites are limited to reproducing in drone[6] brood, leading to a stable host-parasite relationship (Locke 2016). Similar hygienic behaviors can be produced in Western honey bees (*Apis mellifera*) through selective breeding, but selection pressure must be maintained continually (Erickson, Atmowidjojo and Hines 1998). Selection pressure includes "requeening" colonies with varroa-resistant queens and eliminating susceptible colonies while mating queens only with drones from other varroa-resistant colonies. Beekeepers do not always adopt such stocks due to historical preferences for specific varieties, or valuing production or bee gentleness over varroa mite resistance (APHIS 2014). Additionally, because some beekeepers transport bees from one region to another, one stock of bees may not have the characteristics necessary for all aspects of their operation. Western honey bees that have been bred for mite resistance often form smaller colonies and have lower honey production (Locke 2016).

Three active defense traits by bees that support varroa resistance are:

- mite non-reproduction (suppression of, or otherwise reducing mite fecundity);
- removal of mite-infested brood by workers; and
- varroa sensitive hygenic behaviors (VSH), whereby bees remove mites when grooming, sometimes resulting in mites with chewed appendages (APHIS 2014, Locke 2016, Rinderer, et al. 2010).

While many "survivor bees"[7] are not as productive from a beekeeper's perspective, resistant strains have been developed—including Russian honey bees, Minnesota Hygienic honey bees, and those from Le Rucher D'Oc—which have been used successfully on bee operations (APHIS 2014). In some cases, these strains have allowed beekeeping operations to forego miticides for more than ten years. Purdue University developed honey bee populations over the course of six years that increased mite chewing behavior during grooming from 3 percent to 44 percent (APHIS 2014). In Sweden, honey bees artificially infested with varroa mites on the island of Gotland were left to evolve on their own. After three years, over 80 percent of the colonies died, but subsequently the mite infestation rate and the winter honey bee mortality rate decreased. These bees developed a characteristic which reduced the mite population growth rate by 82 percent (APHIS 2014, Locke 2016). Based on a review of genetic studies, Locke speculated that this characteristic could be a volatile substance produced by bees that influences mite egg production.

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[6] Drones are male bees whose sole task is to mate with honey bee queens. They are larger than worker bees (Penn State 2011).

[7] "Survivor bees" are those from colonies that exhibit resistance or tolerance under varroa mite pressure.

Russian, Minnesota Hygienic, and VSH bees are commercially available (Foley's Russian Bees 2018, B and B Honey Farms, LLC 2018, Wildflower Meadows, Inc. 2018).

Floor screens

Adding screens that allow mites, but not bees, to pass through can be used to delay colony infestations (APHIS 2014). Harbo and Harris (2004) found that hives with an open screen floor had fewer mites (1.4 mites/bee vs. 2.5 mites/bee), 13–21 percent lower proportion of mites within brood cells, and 10–17 percent more capped brood, without negative effects on bees. The screens appeared to increase the amount of time between when mites left one brood cell and entered a new one (9.4 days vs. 4.4 days). Delaplane et al. (2005) found similar results for screened floors but also observed a 31 percent reduction of honey and 30 percent reduction of pollen stores per colony as compared with solid floors.

Dusting

Dustings with a variety of materials (e.g., powdered sugar, ground pollen, wheat flour) may adhere to specialized structures on mites called ambulacra, preventing phoretic mites (those that are outside of brood cells) from attaching to the surface of honey bees (Fakhimzadeh 2001). Powdered sugar applications may also stimulate honey bee grooming, which in turn leads to mite fall (Rinkevich, Danka and Healy 2017).

Rinkevich et al. (2017) found that mite mortality from powdered sugar treatments combined with screened bottom boards was not different from untreated groups throughout the entire season, but mite infestation was slowed in the months of August and September compared with controls. Fakhimzadeh (2001) found that under field conditions, mite fall due to powdered sugar treatments was significantly higher than in control groups. Macedo and Ellis (2002) found similar results but recommended the use of powdered sugar dusting as a monitoring tool, rather than as a control measure. Aliano and Ellis (2005) developed a process whereby adult bees were driven from the hive into a detachable box using a bee repellent, and subsequently powdered sugar was applied. The process removed 76 percent of phoretic mites from the honey bees.

Brood removal

Mites preferentially infest drone brood over worker brood (Aliano 2008; Rinderer, et al. 2010). Infested drone brood combs can be removed without negative effects on colony size or honey production, and this can reduce final mite population by 50-70 percent (Rosenkranz, Aumeier and Ziegelmann 2010). Alternatively, brood "traps" can be employed; in a broodless colony, a frame of open (unsealed) brood cells is added. Mites move to the brood cells to reproduce. These cells are then capped, and the brood combs with the mites are removed, potentially resulting in more than 90 percent mite reduction (DEFRA 2017; de Ruijter 1999). Brood traps can also be combined with artificial swarms: The queen is housed/caged in a new hive that is still in the old location, with the old hive moved some distance away. As worker bees from the old hive return from foraging, they move to the new hive. After three weeks, all brood in the old hive are hatched, and brood traps are placed (DEFRA 2017). This process allows the beekeeper to simultaneously control the brood cycle (and therefore increase the effectiveness of the brood traps) and honey bee swarming behavior.

Heat treatment

Heat treatment, or thermotherapy, requires specialized hive structures such as Linhart's "Thermosolar Hive"[8] and "Be Climatized Hive-Solar"[9] or heaters such as "The Victor-For Varroa Mite Thermal Treatment."[10] The treatment is made possible by the difference in heat tolerance between varroa mites and honey bees. Varroa mites prefer temperatures between 26–33°C (Rosenkranz, Aumeier and Ziegelmann 2010). Over 37–38°C and 40–70 percent relative humidity (RH), varroa mite female fecundity is no longer observable, while the same conditions do not affect bee mortality (Le Conte, Arnold and Desenfant 1990). In brood cells at 40 percent RH and 38°C, varroa mite mortality reaches 100 percent after 24 hours. In one study, heat treating capped brood for 150 minutes at temperatures between 40–47°C resulted in nearly

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[8] Thermosolar Hive, http://thermosolarhive.com/en/homepage/

[9] Bee Ethic, https://www.beeethic.com/

[10] Green Beehives, http://www.greenbeehives.com/thevictor.html

100 percent mite mortality, with no observable effects on bees or comb wax (Bičík, Vagera and Sádovská 2016). Similarly, a study conducted on caged and heat treated adult bees demonstrated that after 48h and 40°C, all mites had fallen off their host (J. R. Harbo 2000). Harbo noted that heat treatment is risky as a technique, because the therapeutic temperatures are close to those that are damaging to bees.

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