

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:	18	
Oxalic acid (incl. anhydrous and dihydrate forms)	19	
Ethanedioic acid (incl. anhydrous and dihydrate forms)	20	
C ₂ H ₂ O ₄	21	Trade Names:
(COOH) ₂	22	Oxalic acid dihydrate
C ₂ H ₂ O ₄ · 2(H ₂ O)	23	Api-Biocal
(COOH) ₂ · 2(H ₂ O)	24	
		CAS Numbers:
		144-62-7
		6153-56-6 (dihydrate)
Other Names:		
OAD		
Oxiric acid		Other Codes:
Ethanedionic acid		UNII: 0K2L2IJ590
Acidum oxalicum		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.*

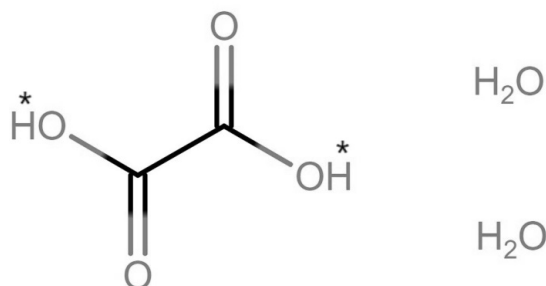
Unless otherwise specified, this report uses the terms “honey bee(s)” or “bee(s)” to refer to the Western honey bee species *Apis mellifera*. The terms “varroa mite(s)” or “mite(s)” are used to refer to the species *Varroa destructor*.

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

57 **Composition of the Substance:**

58 Oxalic acid (OA) is the smallest dicarboxylic acid, composed of a bonded pair of carboxyl groups (see
59 Figure 1 below) (King and Korter 2010). When solid, it exists in two crystalline forms: anhydrous oxalic acid
60 (AOA) and oxalic acid dihydrate (OAD). Pure OAD is 71.42 wt % oxalic acid and 28.58 wt % water
61 (Riemenschneider and Tanifuji 2011). When dissolved, OA is no longer in a crystalline form and therefore
62 neither anhydrous nor a dihydrate.
63



64 **Figure 1: Chemical structure of oxalic acid dihydrate (OAD), adapted from the National Library of**
65 **Medicine (U.S. National Library of Medicine 2018a). Asterisks note where protons (hydrogen ions) can**
66 **be lost, forming either an acid oxalate (loss or exchange of only one proton) or an oxalate (loss or**
67 **exchange of both protons).**
68
69

70 **Source or Origin of the Substance:**

71 *Chemical sources*

72 On an industrial scale, OA is produced as the dihydrate (OAD) via chemical reactions through numerous
73 routes, including oxidation of carbohydrates and other organic compounds using nitric acid (NCBI 2018;
74 Sullivan, et al. 1983; Rohl and Knepper 1975; Riemenschneider and Tanifuji 2011), heating sodium formate
75 in the presence of sodium hydroxide or sodium carbonate (Walter 1996; NCBI 2018; Riemenschneider and
76 Tanifuji 2011), and reacting carbon monoxide with butanol (Fenton and Steinwand 1968; Cassar and
77 Gardano 1978; Riemenschneider and Tanifuji 2011). These manufacturing processes are discussed in detail
78 under *Evaluation Question #2*.
79

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81 Commercial OA is produced from chemical sources. According to a summary of market data published in
82 2008, 124,000 tons of OA were produced from chemical sources, with no known microbial sources (Sauer, et
83 al. 2008). China is the leading producer of OA, while the United States is not known to produce any (Kharas
84 2014). Kharas (2014) states that the annual consumption in the United States (8,000 tons) and annual global
85 consumption (115,000 tons) adds up to a total market value of \$1.1 billion per year.
86

87 *Natural sources*

88 Naturally occurring OA (e.g., in plants, fungi, bacteria, and animals) exists in salt forms such as potassium,
89 calcium, or other oxalates (Riemenschneider and Tanifuji 2011). It can be a considerable constituent of the
90 dry weight of some plants, including beet leaves (12 percent), cocoa (4.5 percent), tea (3.7 percent), spinach
91 (3.2 percent), rhubarb (2.4 percent), and chard (0.69 percent) (Riemenschneider and Tanifuji 2011). It occurs
92 naturally in honey at concentrations between 8–300 mg/kg (Charrière and Imdorf 2002) and is also
93 produced in the human body via the metabolism of glyoxylic acid or ascorbic acid (NCBI 2018).
94

95 In microorganisms, OA is produced through two biochemical pathways that oxidize carbohydrates via the
96 citric acid cycle or variants thereof (Kobayashi, et al. 2014). Some organisms may also produce OA to bind
97 and detoxify metals such as aluminum and copper (Hamel, Levasseur and Appanna 1999; Green III and
98 Clausen 2005). In the fungal pathogen *Sclerotinia sclerotiorum*, OA damages plant cells and inhibits plant
99 defense responses (Durman, Menendez and Godeas 2005). Similarly, in the industrially important fungus
100 *Aspergillus niger*, OA may be involved in degrading plant cell walls to mobilize nutrients (Mai, Lee and
101 Choi 2016).
102

103 *Other sources*

104 OA can exist in the atmosphere from both natural and synthetic sources. It is a component of car exhaust,
 105 for instance, but may also form naturally from acetylene or ethene in the aqueous phase of clouds (Crahan,
 106 et al. 2004; Warneck 2003). OA is the most abundant dicarboxylic acid in aerosols¹ in the troposphere,
 107 ranging from 10-50 ng/m³ in remote regions to 900 ng/m³ in urban air (Warneck 2003).

108
 109 **Properties of the Substance:**

110 OA exists as both a solid and as a vapor (i.e., gas). When the solid is heated above its melting point (see
 111 Table 1) but below its decomposition temperature, it will sublime² to the gaseous state (Hussain, Khan
 112 and Shabeer 2012; Riemenschneider and Tanifuji 2011). The dihydrate solid, OAD, contains two molecules
 113 of water of crystallization; dehydrating OAD (e.g., through heating) will yield the anhydrous form AOA
 114 (Walter 1996; Riemenschneider and Tanifuji 2011).

115
 116 OA molecules are arranged in flat sheets or chains within the crystal, with the carboxyl groups oriented in
 117 the trans conformation (i.e., facing opposite directions) with respect to each other as shown in Figure 1 on
 118 the previous page (Godfrey, Mirabella and Brown 2000). OA crystals will dissolve in polar solvents such as
 119 water or alcohol (Hussain, Khan and Shabeer 2012), though alcohols will also partially react with OA to
 120 form oxalate esters (Riemenschneider and Tanifuji 2011).

121
 122 OA is a strong acid that forms metal-oxalate salts (Riemenschneider and Tanifuji 2011). It is dibasic, being
 123 able to donate protons (hydrogen ions) from two separate acidic groups. The nearness of the two acidic
 124 groups leads to an increase in the strength of acidity; OA has a dissociation constant (K₁) comparable with
 125 some mineral acids, including sulfurous acid. The salts are either acid oxalate (i.e., a single cation
 126 replacement) or normal salts (i.e., where both acidic groups are replaced with cations) (Walter 1996). OA
 127 acts as a reducing agent (American Chemical Society 2006), decomposing into carbon dioxide and water
 128 (Riemenschneider and Tanifuji 2011). While OA is soluble in water at neutral pH, oxalate salts such as
 129 calcium oxalate are not (with the exception of alkali metal and iron (III) salts) (Hussain, Khan and Shabeer
 130 2012; Riemenschneider and Tanifuji 2011).

131
 132 **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

133 ^aSources: (American Chemical Society 2006; Walter 1996; LabChem 2018; Science Lab.com 2013; O'Neil, et
 134 al. 2013; Hussain, Khan and Shabeer 2012; Riemenschneider and Tanifuji 2011)

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 sublime do not transition to a liquid phase in the process of melting.

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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 period³ 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 sublime (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 dm² (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)

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).

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

186

187 *Other uses*

188 OA is a reducing agent that can be used as an analytical reagent and standard, a wood bleach, a metal
189 polish, and a metal precipitating or chelating agent (Walter 1996). It is used as a stain remover and mordant
190 in the textile industry (Cassar and Gardano 1978; CAMEO 2016; Zaher, Fritzler and Hutchinson 2005; NCBI
191 2018; Vishwakarma and Gogate 2011) and as a cleaning and sterilizing agent that removes calcium in water
192 treatment applications (Vishwakarma and Gogate 2011). It has reportedly been used to hydrolyze corncobs
193 as a pre-treatment in biofuel production (Lee, et al. 2011) and applied as an environmental control for ticks
194 (Valcárcel, et al. 2014). Reimenshneider and Tanifuji (2011) quantified its principal applications as
195 approximately 28 percent metal treatment, 25 percent textile treatment, 20 percent bleaching agents, and
196 27 percent chemical uses. The petitioned use was not specifically addressed in these estimates but may be
197 considered under the category of chemical uses.

198

199 **Approved Legal Uses of the Substance:**

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

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

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

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

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

219

220 The FDA permits the use of OA as an indirect additive used in food contact substances under 21 CFR
221 177.1010 (for use as a polymerization catalyst aid in polymers used as basic components of single and
222 repeated use food contact surfaces) and under 21 CFR 177.2410 (as an optional catalyst in the production of
223 phenolic resins).

224

225 **Action of the Substance:**

226

227 *On mites*

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

238
239 One study evaluated the action of different OAD treatments: by sublimation (1 g OAD per hive), oral
240 application (70 µg per bee) and topical application (70 µg per bee) to the abdomen of phoretic mites. The
241 concentrations approximate what would typically be used in a hive (Papežíková, et al. 2017). The study
242 found OAD crystals attached to the bodies of dead mites, even for those with brief exposure of five
243 minutes. Only around 12 percent of mites displayed cuticular damage as observed under a dissecting
244 microscope. Besides two fatally damaged mites – which had missing legs and a split dorsal shield – the
245 majority that sustained cuticular damage had only mild compression of the dorsal shield, which would not
246 be expected to affect viability. These findings support the hypothesis that OA acts via contact toxicity on
247 varroa mites rather than the OA crystals causing structural damage. The authors speculated that OA may
248 affect the mite’s ability to hold onto the bee’s body and/or its ability to re-infest the host. The group found
249 that oral treatment of bees with OA also caused mite mortality, despite there being no direct contact of the
250 mites with OAD crystals. The authors suggested that this could be due to metabolic disturbances and
251 changes in the bees’ hemolymph affecting the survival of the mites, which are tightly adapted to their hosts.
252

253 Mites are likely unable to detect OA by olfaction⁴ (Papežíková, et al. 2017), and Ailano et al. (2006)
254 surmised that inhalation of OA vapors is not a significant factor in the substance’s toxicity to mites due to
255 its low volatility at room temperature. However, with the sublimation method, mites would be exposed to
256 OA in the gas phase until it crystallizes on their bodies.
257

258 *On honey bees*

259 While honey bees are more tolerant to OA than mites, they do exhibit some negative effects, especially at
260 higher concentrations. When treated with doses insufficient to directly cause mortality (i.e., sub-lethal
261 doses), bees experience decreased activity and changes to cuticle (exoskeleton) enzymes. Doses large
262 enough to cause mortality cause internal tissue damage (Martín-Hernández, et al. 2007). Additional details
263 on various effects are described here and in *Evaluation Question #5*.
264

- 265 • *Histopathological (tissue) effects:* Martín-Hernández et al. (2007) conducted histopathological tests
266 using direct topical application of an OA solution (presumably OAD) at 660 µg/bee, which did not
267 cause visible external damage to the subjects. However, it caused progressive damage to internal
268 organs, including epithelial tissues of the ventriculus, Malpighian tubes, and rectum, which
269 increased for at least 72 hours – the length of the experiment. The increased cellular damage over
270 time suggests that the effect of OA continues after initial contact and, according to the authors, may
271 cause permanent lesions. The authors also suggested that some OA may be ingested by bees during
272 grooming (Martín-Hernández, et al. 2007). Radermacher et al. (2017) found that dermal application
273 of OAD to honey bees is relatively well tolerated, but oral application is three times more toxic.
274 Larvae subjected to a spray application of 2.97 percent OAD exhibited minor damage to the midgut
275 (Gregorc, Pogacnik and Bowen 2004). Papežíková et al. (2017) found that trickling OAD solutions
276 increased the rate of apoptosis in bees’ midgut cells, suggesting cell damage, though food intake
277 did not differ between treatment groups. In contrast to the findings of Martín-Hernández et al.
278 (2007), Papežíková et al. did not find epithelial destruction in Malpighian tubules, possibly because
279 there was less direct exposure via the trickling method in their study compared to direct
280 application in the former.
281
- 282 • *Biochemical effects:* The glutathione S-transferase family of enzymes is common to most organisms,
283 and serves to convert reactive molecules, including carboxylates, into non-reactive forms for the
284 purpose of detoxification (Clark 1989). Nanetti et al. (1999) reported no difference in glutathione S-
285 transferase (GST) activity between treated and untreated groups of bees, which led the authors to
286 conclude that OA trickling at normal dose does not compromise the bee digestive system or
287 weaken the detoxifying activity against potentially harmful substances. However, a study in
288 Poland assessed the effects of oxalic acid treatment on bees’ cuticle proteolytic enzymes (CPEs),

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).

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

- 295
296 • *Sublethal effects:* Bees exhibit behavioral changes when treated via trickling at sub-lethal doses of
297 OA. At 175 µg OAD/bee, Schneider et al. (2012) found that the treatment caused a decrease in
298 worker activity, nursing behavior, and longevity, but caused an increase in grooming behavior
299 (2012). Neurotoxic damage was suspected as one possible cause of the change in behavior based on
300 similar results from another study where bees treated with formic acid were immobilized and did
301 not regain mobility. However, the authors also acknowledged that the changes in bee behavior
302 could have resulted from damage to the digestive and excretory organs, causing an insufficient
303 resorption of nutrients through the epithelium of the gut and resultant weakening of the bees. For
304 comparison, the dosage used in this study was roughly three times higher than the typical
305 maximum dosage of 57 µg OAD/bee for a honey bee colony of 35,000 using the trickle method of
306 application (Aliano, Ellis and Siegfried 2006).

307 308 *Variables*

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

- 313
314 • *Concentration:* Variations in mite mortality with different concentrations of OAD suggest contact
315 toxicity (Milani 2001). One study used a 3 percent OAD concentration at a dosage of 3 µL per bee –
316 roughly equivalent to 5 mL per seam – with an efficacy rate of 91.3 percent. The amount of OAD
317 applied to each bee was about 90 µg (Rademacher and Harz 2006). Efficacy increased to
318 98.4 percent when the concentration was increased to 4.6 percent OAD. However, higher
319 concentrations (e.g., 6 percent OAD) did not necessarily increase mite mortality and posed a greater
320 risk for bees. A similar volume applied to twice as many bees (3.0 and 1.5 µL, respectively) with the
321 concentration doubled from 2.3 to 4.6 percent OAD resulted in 98 percent efficacy and 80 percent
322 mite fall (Büchler 2000, reviewed in Rademacher and Harz 2006). Charrier et al. (2015) also noted
323 that concentration can be varied to optimize efficacy depending on climatic region.
- 324
325 • *Frequency:* Repeated applications of OAD result in a higher level of bee mortality (Rademacher and
326 Harz 2006).
- 327
328 • *Time dependence:* Martín-Hernández, et al. (2007) observed increased cellular damage to bees over
329 time following OA treatment. The pathological effects observed after 48 hours were severe and
330 irreversible, leading the authors to conclude that OA continues to act on organisms following initial
331 contact and that OA exposure can cause permanent lesions that may be time- and dose-related.
- 332
333 • *Climate and season:* It is widely acknowledged that oxalic acid treatments are most effective during
334 broodless periods because OA does not affect varroa mites in capped brood cells (Gregorc and
335 Planinc 2001; Bacandritsos, et al. 2007). For this reason, application is recommended for late fall or
336 early spring in temperate climates when brood are most often absent. OA efficacy is greatly
337 reduced in warmer climates where brood rearing periods are much longer (Higes, Meana and
338 Suárez 1999; Rademacher and Harz 2006; Higes, Martín-Hernández and Meana 2006). According to
339 the EPA-registered label for OAD, application of OAD when brood is present might also damage
340 the brood (EPA 2015c).
- 341
342 • *Formulants and relative humidity:* Sugar and, to a lesser extent, glycerol used in combination with OA
343 appear to have a synergistic effect in increasing mite mortality. Milani (2001) demonstrated that

344 neither sucrose or glycerol increased mortality over controls on their own, indicating that they
345 improved dispersability or had some other physical effect on OA treatments. The study noted that
346 with both formulants the OA absorbed water at lower relative humidity, and the resulting
347 concentrated liquid deposits of OA were more effective than dry deposits. For more details on
348 these formulants, see *Combinations of the Substance*.

349

350 **Combinations of the Substance:**

351 The petition for oxalic acid lists ancillary substances as “not applicable” (Domeier 2017). Specifications for
352 impurities in OAD as a chemical reagent are: no more than 0.005 percent insoluble matter or sulfate,
353 0.002 percent chloride, 0.001 percent calcium or nitrogen compounds, and no more than 5 ppm heavy
354 metals, or 2 ppm iron (American Chemical Society 2006).

355

356 The label for EPA Registration No. 91266-1 lists OAD as 97 percent of the product composition, with
357 3 percent inert ingredients. Information regarding the identity of the inert ingredients is not publicly
358 available.

359

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

365

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

373

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

377

378

379

Status

380

381 **Historic Use:**

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

385

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

396

397 Organic Foods Production Act, USDA Final Rule:

398 OA does not appear in the Organic Foods Production Act or in the USDA organic regulations, 7 CFR Part
399 205.

400

401 International

402

403 *Canadian General Standards Board Permitted Substances List*

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

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

413

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

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

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

425

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

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

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

433

434 *Japan Agricultural Standard (JAS) for Organic Production*

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

438

439 *IFOAM – Organics International*

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

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

446

447

448

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

449

450 **Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the**
451 **substance contain an active ingredient in any of the following categories: copper and sulfur compounds,**

452 toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed,
453 vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree
454 wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance
455 a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA
456 List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on
457 EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

458

459 A) OA is a livestock parasiticide.

460 B) OA is a not an inert ingredient.

461

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

466

467 Although the available literature confirms that commercially available sources of OA are chemical sources,
468 the specific manufacturing process used to manufacture the OAD ingredient used in EPA-registered varroa
469 mite control products is unknown. Several authors (Riemenschneider and Tanifuji 2011; Betiku, Emeko and
470 Solomon 2016; Mandal and Banerjee 2005) state that OA is usually produced through the oxidation of
471 carbohydrates or alkenes (including ethylene or propene), or synthesis from carbon monoxide and water.
472 Other methods may still be used by small firms, including the sodium formate method, alkali fusion of
473 cellulose, and the isolation of oxalic acid as a byproduct of fermentation (Riemenschneider and Tanifuji
474 2011). Many methods share similar features: a) organic (carbon-containing) molecules are oxidized with
475 nitric acid and catalysts to form oxalic acid; b) organic molecules are oxidized and form salts, which are
476 then purified using sulfuric or hydrochloric acid to form OA and calcium sulfate or sodium chloride
477 (Zaher, Fritzier and Hutchinson 2007); or c) OA is synthesized from smaller molecules like carbon dioxide
478 or carbon monoxide.

479

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

485

486 Chemical sources (common methods):

487 • *Carbohydrate oxidation:* Carbohydrates from a variety of sources are hydrolyzed to glucose
488 monomers by boiling in oxalic or sulfuric acid. Glucose is then reacted with nitric acid in the
489 presence of vanadium pentoxide and iron to form OA, nitrogen oxides (which are recovered), and
490 water (Fuchs and Watson 1970). OA may be produced this way in Spain, Brazil, China, Taiwan,
491 Korea, and India (Riemenschneider and Tanifuji 2011).

492 • *Ethylene glycol oxidation:* Ethylene glycol is reacted with nitrogen tetroxide and oxygen or nitric acid
493 and sulfuric acid to form OAD in a one-step process (Young 1924; Yonemitsu, et al. 1972). In the
494 Mitsubishi Gas Company process, carbon dioxide is the primary byproduct, and catalysts such as
495 vanadium pentoxide are not required (Yonemitsu, et al. 1972). According to Riemenschneider &
496 Tanifuji (2011), most of the OA produced in Japan uses this method.

497 • *Propene oxidation:* Propene, generated from petrochemical refinement, is partially oxidized with
498 nitric acid to form α -nitratolactic acid, lactic acid, nitrous oxide, and water. The α -nitratolactic acid
499 is reacted with oxygen and catalysts (such as chromium or iron nitrate and tin chloride) to form
500 oxalic acid, carbon dioxide, nitric acid, and water. This process can form unstable intermediates
501 that can lead to violent explosions, and is performed by only one company, located in France
502 (Riemenschneider and Tanifuji 2011).

503 • *Synthesis from carbon monoxide and water:* Carbon monoxide is reacted with alcohol (such as butanol)
504 under pressure, using a platinum or palladium salt and ferric or cupric chloride salt as catalysts.
505 Then, either oxygen is added, or a DC electric current is used to maintain an oxidized state, to keep
506 the salts functioning catalytically (Fenton and Steinwand 1968). In the first step, an OA diester is

507 formed from the carbon monoxide and alcohol, which is then hydrolyzed to form OA and
508 regenerate the original alcohol (Riemenschneider and Tanifuji 2011). The method can vary through
509 additions of ammonia (Cassar and Gardano 1978).
510

511 Chemical sources (other less common methods):

- 512 • *Synthesis from carbon dioxide and water:* Kharas (2014) and Twardowski et al. (2016) developed
513 methods whereby carbon dioxide and water are electrochemically converted to OA using
514 electrolyzed water, created using electrochemical catalysts such as cobalt and a platinum-group, or
515 using similar metal and a lithium salt.
- 516 • *Synthesis from sodium formate:* Carbon monoxide is absorbed into a heated solution of sodium
517 hydroxide under pressure to form sodium formate, which is then evaporated and heated again to
518 form sodium oxalate, sodium carbonate, and hydrogen gas. The sodium oxalate is treated with
519 calcium hydroxide and water to precipitate calcium oxalate and simultaneous regeneration of
520 sodium hydroxide. The calcium oxalate is treated with sulfuric acid to form OA and calcium sulfate
521 (Wallace 1926). Alternatively, sodium oxalate can be treated with nitric acid to form OA and
522 sodium nitrate (Beckham 1954). Riemenschneider & Tanifuji (2011) note that some of the steps
523 involved with the sodium formate method are cumbersome and are of questionable safety but may
524 still be used in China.
- 525 • *Alkali fusion of cellulose:* Cellulosic material such as sawdust is reacted with alkali such as sodium
526 hydroxide under pressure, which forms sodium oxalate (Lidbury 1912; Bannister 1934; Krochta
527 1989). Bannister (1934) speculated that the cellulosic material first decomposed into formic acid,
528 and then formic acid decomposed further to OA. Sodium oxalate is converted to OA through
529 treatments with sulfuric acid, similar to that used in the sodium formate method (Riemenschneider
530 and Tanifuji 2011). The Merck Index (O'Neil, et al. 2013) notes that the alkali fusion method has
531 been replaced by other methods.
532

533 Natural sources:

- 534 • *Microbial fermentation:* As reported by Sauer et al. in 2008, existing market data did not show that
535 microbial fermentation sources were used for OA. In contrast, Riemenschneider and Tanifuji (2011)
536 stated that OA may be produced in some parts of the world as a fermentation byproduct during the
537 manufacture of other substances such as citric acid, but the process required large amounts of
538 water and was not economically feasible at that time. Since then, Kobayashi et al. (2014) genetically
539 transformed a strain of *Aspergillus niger* which overexpressed a single enzyme – oxaloacetate
540 hydrolase (encoded by the oahA gene) – and hyperproduced OA in liquid media. This strain
541 produced a yield of 96.3 percent OA based on the glucose consumed. In a report from 2016, a strain
542 of *A. niger* was developed through artificial selection, which produced 123 g of OA/kg dry weight
543 of corncob using a solid-state fermentation process (Mai, Lee and Choi 2016). There is additional
544 interest in further development of OA production through microbial fermentation (Nakata and He
545 2010; Emeko, Olugbogi and Betiku 2015; Mai, Lee and Choi 2016; Betiku, Emeko and Solomon
546 2016).
547

548 **Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a**
549 **chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).**
550

551 Based on available literature, all commercially available OA is produced through chemical processes
552 (Riemenschneider and Tanifuji 2011; Betiku, Emeko and Solomon 2016; Mandal and Banerjee 2005),
553 including the oxidation of larger molecules using acids, or the synthesis of smaller molecules using
554 pressure and catalysts. These processes are detailed above in *Evaluation Question #2*.
555

556 Riemenschneider and Tanifuji (2011) speculated that some developing nations might use fermentation to
557 produce OA, but the market data summarized by Sauer et al. (2008) did not show such processes are used.
558 Although there is interest in producing OA through microbial fermentation, and yield is improving
559 dramatically in some cases (Nakata and He 2010; Emeko, Olugbogi and Betiku 2015; Mai, Lee and Choi
560 2016; Betiku, Emeko and Solomon 2016), no sources positively identified fermentation as a current
561 commercial production method.

562

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

565

566 Naturally occurring OA is ubiquitous in the environment. It has low persistence and no potential for
567 bioaccumulation in the food chain (Chem One Ltd. 2015). OA released into the soil under environmental
568 conditions (ie., pH 5–9) is found in the oxalate ion form and readily biodegrades (U.S. National Library of
569 Medicine 2005; Environment Directorate General of the European Commission 2014). OA begins
570 biodegradation under anaerobic and aerobic conditions in less than one day (EPA 1992). One Material
571 Safety Data Sheet for commercial OA states aerobic biodegradability as being 89 percent after 20 days
572 exposure time and classifies this as being readily biodegradable (Penta International Corp. 2018). Aerobic
573 and anaerobic biodegradation are the predominant fate of OA in subsurface waters. OA does not volatilize
574 at room temperature, adsorb to sediment, bio-concentrate in aquatic organisms, or oxidize or hydrolyze in
575 water under natural conditions. In surface waters and on soil surfaces, OA primarily undergoes photolysis
576 (breakdown by light). Daytime persistence of OA on soil surfaces is expected to be less than a few hours. In
577 the atmosphere, the amount of OA that can be supported by cloud water is 9–18 ng m³ for short-lived
578 clouds, and 28–56 ng m³ for long-lived clouds where the chemistry stabilizes (Warneck 2003). The
579 atmospheric fate of OA is likely to be wet or dry deposition, followed by biodegradation as described. OA
580 may also undergo photolysis in the atmosphere (U.S. National Library of Medicine 2005).

581

582 *Degradation methods and by-products*

583 In all its degradation pathways, OA primarily decomposes into carbon dioxide and water, and to a lesser
584 extent carbon monoxide and water. As a reducing agent, OA reacts readily with oxygen, yielding carbon
585 dioxide and water (Riemenschneider and Tanifuji 2011). Under normal circumstances, the decomposition
586 temperature of OAD is 157°C (LabChem 2018), at which point it decomposes into carbon monoxide, carbon
587 dioxide, and water with formic acid as an insoluble intermediate (Riemenschneider and Tanifuji 2011). The
588 decay of OA to carbon dioxide may also be biologically or enzymatically mediated, as happens to OA in
589 nature with white rot and brown rot fungi depolymerizing wood hemicellulose (Espejo and Agosin 1991).
590 AlSalka, et al. (2018) reported that in aqueous solutions, gamma- or X-rays induce decomposition of OA to
591 carbon dioxide and hydrogen at a molar ratio of 2:1 and generate carbon monoxide and formic acid. The
592 authors observed complete photocatalytic degradation of OA into carbon dioxide and hydrogen within
593 60 minutes of illumination under aerobic conditions with no byproduct detected and with only minor
594 formation of formic acid under anaerobic conditions (AlSalka, et al. 2018). OA is convertible to formic acid
595 and vice versa (Walter 1996).

596

597 *Residues of OA in honey*

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

613

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

617
618 The mode of action of OA on both varroa mites and honey bees is discussed above under *Action of the*
619 *Substance*. The persistence and areas of concentration of OA and its degradation byproducts are discussed
620 above in *Evaluation Question #4*. OA is classified as a hazard class 1, or low danger, for water and has low
621 toxicity for fish and bacteria (Riemenschneider and Tanifuji 2011). It has been reported to inhibit the activity
622 of some bacteria during natural interactions between fungi and plants (Nagarajkumar, et al. 2005).

623
624 *Toxicity of OA to honey bees*

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

632
633 Papežiková et al. (2017) examined the effect of OAD treatment on bees' lifespan, finding that lifespan had
634 not significantly decreased 21 days after OAD treatment by sublimation, but that it had significantly
635 decreased with the trickling method. Another study examining longer-term effects found that OA
636 treatment of beehives resulted in decreased numbers of brood and some queen death during the spring
637 following autumn application, a time lapse of 3–4 months (Higes, Meana and Suárez 1999). Papežiková et
638 al. (2017) noted the possibility of delayed toxic effects of OA treatment on bees, and suggested these be
639 further examined in overwintering colonies. Some studies report a loss of about 1,000–2,000 bees, or about
640 6 percent of a typical overwintering colony in temperate field conditions (Charrierre and Imdorof 2002).

641
642 The lethal dose of OAD to 50 percent of a bee population after 72 hours, or the 72-hr LD₅₀, was assessed in
643 several studies. Martín-Hernandez et al. (2007) found the 72-hr LD₅₀ to be 530 µg per bee. Another study
644 estimated the 72-h LD₅₀ for bees to be lower, at 195 µg OAD per bee (Aliano, Ellis and Siegfried 2006). In the
645 latter study, doses of less than 100 µg OAD per bee did not cause significant mortality after 48 hours (48-h
646 LD₁₀ 176.68 µg OAD per bee). Significant mortality occurred only after 72 hours following treatment. The
647 EPA-registered label for OAD specifies a dosage of 35 g OAD dissolved in 1 liter of solution and applied at
648 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
649 average, which is below the reported 72-hr LD₅₀ levels. Rather, the applied dosage is about one-third the
650 chronic toxicity of 175 µg OAD/bee reported by Schneider et al. (2012) and about one-fourth the LD₅₀
651 reported by Aliano et al. (2006). However, Aliano applied doses of OAD to individual bees in acetone, and
652 the acetone may have contributed to the toxicity.

653
654 *Toxicity of degradation by-products*

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

660
661 **Evaluation Question #6: Describe any environmental contamination that could result from the**
662 **petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).**

663
664 The methods for manufacturing OA involve numerous chemicals (see *Evaluation Question #2*). In the
665 carbohydrate oxidation process, vanadium pentoxide – a naturally occurring metal compound – is used as
666 a catalyst. Vanadium pentoxide is considered an air pollutant, predominantly created from oil combustion
667 (WHO 2001). Its use as a catalyst may present health risks in industrial settings. Inhalation of vanadium
668 dust can cause lung irritation and adverse respiratory effects; OSHA limits exposure to 0.05 mg/m³ as an
669 8 hour total weight average (NIOSH 2011). It is not clear whether environmental exposure results from the
670 use of vanadium pentoxide in the manufacturing of OA. Nitric oxides, co-products of OA, are recovered in
671 the process.

672
673 The ethylene glycol method produces carbon dioxide as a byproduct. In the synthesis of OA from carbon
674 monoxide and water, butanol is used and recovered in the process. Other chemicals including metals, salts
675 acids, and bases are used in the various processes. Despite this, no information was found in the literature
676 specifically identifying potential environmental contamination hazards from the manufacturing of the
677 substance.

678
679 The use of OA in honey bee hives has been cited for its low potential for environmental contamination due
680 to OA's high degradability and the application being limited to within the hive. Charrière and Imdorf
681 (2002) state that there are no environmental residues expected from the repeated application of OAD in
682 hives by the various methods described, including spraying, trickling, and sublimation. While sublimation
683 can produce low levels of OA in the air around hives following application, it is expected that these
684 residues degrade in the environment via the same pathways described in *Evaluation Question #4*.

685
686 Despite its low potential for environmental contamination, OA is a strong acid; Riemenschneider and
687 Tanifuji (2011), therefore, recommended that it should not be disposed of directly into the environment but
688 should first be treated either by incineration or heating with sulfuric acid to convert it to carbon monoxide,
689 carbon dioxide, and water. Small amounts of OA may be treated with potassium permanganate to yield
690 carbon dioxide or neutralized with lime to produce calcium oxide, which is safe for disposal
691 (Riemenschneider and Tanifuji 2011).

692
693 **Evaluation Question #7: Describe any known chemical interactions between the petitioned substance**
694 **and other substances used in organic crop or livestock production or handling. Describe any**
695 **environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).**

696
697 *Direct chemical interactions in apicultural systems*

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

703
704 *Indirect interactions and interactions beyond apicultural systems*

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

712
713 OA plays a role in the pathogenicity of various fungi. In this ecological context, fungal exudates of OA
714 precipitate calcium from the middle lamellae of plants and makes them more susceptible to fungal attack.
715 In nature, calcium oxalate can have a depressive effect on the antibiotic activity of beneficial soil
716 microorganisms such as *Pseudomonas fluorescens* (Nagarajkumar, et al. 2005), a beneficial bacteria used in
717 pest control applications in organic crop production (OMRI 2018). At the same time, some strains of *P.*
718 *fluorescens* can detoxify or catabolize OA.

719
720 In addition to forming both neutral and acid salts, OA reacts with most organic and inorganic bases to form
721 crystallizing complexes, including potassium hydrogen oxalate, ammonium oxalate, and ammonium iron
722 oxalate. OA is incompatible with strong alkalines, strong oxidizers, chlorites and hypochlorites, and
723 combustible materials. It may react rapidly in contact with iron and iron compounds to form ferric oxalate.
724 Contact with silver may form silver oxalate. OAD in solution is corrosive to metals (Chem One Ltd. 2015).

725

726 **Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical**
727 **interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt**
728 **index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).**
729

730 *Possibility of varroa mites to develop OA resistance*

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

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

749
750 High frequency and extended duration of use are other factors that can affect the development of resistance
751 to a substance. Milani (2001) suggested that the prolonged used of OA to treat varroosis could contribute to
752 the emergence of resistance, as even a small increase in mites' tolerance would greatly limit its efficacy
753 because increasing concentrations of OA would be toxic to bees. Milani noted that the high efficacy of OA
754 in the absence of capped brood, its ease of use, and low cost could lead to an increase in OA use and to an
755 accompanying increase in selective pressure on mites.

756
757 Maggi et al. (2017) carried out research to assess the potential development of resistance by varroa mites to
758 OA. Their study examined the susceptibility of mite populations that had been subjected to 64 consecutive
759 OA treatments over eight years compared to populations that had never been exposed to OA. They found
760 that the mites that had received ongoing OA treatment remained susceptible to OA, which demonstrated
761 that the varroa mites did not develop resistance to OA despite repeated exposure. Several other researchers
762 have surmised low probability for mites developing resistance to OA (A. Gregorc 2005; Papežíková, et al.
763 2017), stating that the selection pressure should be low due to OA's rapid degradation inside the colony
764 and infrequency of application, especially as compared to synthetic acaricides. At the time of the report by
765 Maggi et al. (2017), no cases of OA resistance had been documented in varroa mites. Nevertheless, the
766 authors did not rule out the potential for resistance development in the future and recommended continued
767 use of precautionary measures to prevent it.

768
769 A different mechanism by which mite populations could develop resistance to OA is through the
770 microflora associated with the mites and/or bees. Maddaloni and Pascual (2015) isolated from *Varroa*
771 *destructor* certain bacteria that possess a rare trait known as oxalotrophy, or the ability to degrade OA by
772 using it as their sole carbon source. If the association with these bacteria helps varroa mites survive OA
773 treatment, that association could expand or increase through selective pressure, thereby leading to lower
774 susceptibility of mite populations to OA treatment in the future (Maddaloni and Pascual 2015). The
775 literature review for this report did not identify any documented cases of this phenomenon to date.

776
777 **Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned**
778 **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)**
779 **(i)).**
780

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

786
787 **Evaluation Question #10: Describe and summarize any reported effects upon human health from use of**
788 **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**
789 **(m) (4).**

791 OA is acutely toxic to humans when ingested, inhaled, or when it comes into contact with the body. It
792 absorbs relatively well through the skin and is easily absorbed through mucous membranes, which can
793 lead to absorptive poisoning (Riemenschneider and Tanifuji 2011). OA is also corrosive to the skin (ILO and
794 WHO 2009). Chronic skin exposure causes localized pain and may lead to dermatitis, slow-healing ulcers,
795 and gangrene. The oral minimum lethal dose in humans (LD_{Lo}) – the lowest amount of OA required to
796 induce death – has been reported as 4–5 g, and elsewhere as 71 mg per kilogram (kg) of body weight
797 (which is equivalent to 4.26 g for a 60-kg person) (Riemenschneider and Tanifuji 2011). The amount of OA
798 applied to each beehive via the vaporizer method, 2 g OAD, is 40 percent of the minimum lethal dose. OA
799 dust or vapors cause irritation or severe burning of the eyes and upper respiratory tract. Signs of chronic
800 exposure via inhalation include chronic coughing, vomiting, and general weakness (Riemenschneider and
801 Tanifuji 2011). OSHA standards limit the permissible exposure to OA to a time weighted average of
802 1 mg/m³ over 8 hours (NCBI 2018).

803
804 OA, while acutely toxic, does not pose risk for any systemic effects within the body (Rademacher and Harz
805 2006). However, in one study, anhydrous OA was tested for biological activity in mice; at a concentration of
806 0.2 percent AOA in drinking water, it was found to reduce water consumption to the extent that the mice
807 had fewer litters per pair, lower pup weight, and decreased prostrate weight in the absence of detected
808 somatic organ changes. Secondary effects of the treatment in the next generation mice were increased
809 kidney weight, fewer live pups per litter, and increased abnormal sperm forms (Lamb, et al. 1997). A study
810 in rats tested the oral median lethal dose (LD₅₀) of OA – the dose required to kill half the members of the
811 tested rat population after a specified test duration. The LD₅₀ for rats was reported as 475 mg per kg of
812 body weight for males and 375 mg per kg of body weight for females (EMEA 2003).

813
814 Notwithstanding its acute toxicity at higher concentrations, OA is a natural minor component of many
815 foods, including plant-based foods and honey. Because OA is naturally occurring in food and is not a food
816 additive, there is not an established Allowed Daily Intake (ADI); however, one study suggested an ADI of
817 0.89 mg/kg, corresponding to an intake of 53.4 mg/day for a 60 kg human (Rademacher and Harz 2006).
818 As discussed under *Evaluation Question #4*, the amount of OA present in honey as a residue after
819 application for varroa treatment in hives is negligible, approximately 60–80 mg/kg (EPA 2015a), and is not
820 expected to exceed the range of naturally occurring OA concentrations found in honey (PMRA 2010). The
821 EPA has reported this range as being 1–800 mg/kg. In comparison, plants are reported to contain from
822 5 mg/kg to 200 g/kg (EPA 2015a; EMEA 2003). The estimated daily dietary intake of OA is estimated to be
823 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
824 consumed (EMEA 2003). This exceeds the suggested ADI of 0.89 mg/kg, though most dietary OA is in the
825 form of salts, which dissolve in the stomach, precipitate again in the colon, and are excreted. Overall, the
826 use of OAD as petitioned is not expected to result in dietary risk from food consumption (PMRA 2010; EPA
827 2015a).

828
829 Health Canada's Pest Management Regulatory Agency (PMRA) evaluated OA for use as a control agent for
830 varroa mites in honey bee hives, and its registration in Canada was approved in 2010. Subsequently, the
831 EPA was able to expedite the registration of OA in the United States due in part to a NAFTA "work share"
832 agreement in which PMRA officials provided data reviews to EPA risk assessors and risk managers (EPA
833 2015a). These registration decisions were based on the assessment of "reasonable certainty that no harm to
834 human health, future generations, or the environment will result from use or exposure to the product under
835 its conditions of registration." The assessment considered unique characteristics of sensitive

836 subpopulations, such as children, as well as organisms in the environment most sensitive to contamination.
837 The agency's conditions for registration of the substance included precautionary measures on the label to
838 reduce risk (PMRA 2010).

839
840 PMRA (2010) acknowledged that exposure to OAD may occur when handling or applying the product in
841 honey bee production. However, they determined that application at the doses specified would keep the
842 level of potential exposure within that considered safe, having no adverse health effects. A study by
843 Gump (2004) provided evidence that OAD, when applied correctly, poses no inhalation risk to the
844 beekeepers' health. Concentrations measured for OA in the air after application by spraying and
845 evaporation were lower for free-standing hives than in bee houses, but both remained below 0.3 mg/m³,
846 which is less than the existing exposure limit for oxalic acid of 1 mg/m³. Rademacher and Harz (2006) also
847 reported that OA concentration in the air surrounding apiaries where multiple systems are used may
848 increase beyond the levels reported above. The authors recommend that colonies not be inspected directly
849 after treatment because data are not available regarding OA concentration in the air of the hive over time.
850 The EPA-approved label for OAD instructs handlers to use personal protective equipment (PPE) and to
851 avoid breathing dust or fumes as well as to avoid contact with eyes, skin, and clothing. It also instructs
852 users to wash hands and PPE directly following use (EPA 2015c).

853
854 Thus, while OA can be safely applied without compromising the health of the applicator, proper safety
855 measures must be followed to avoid adverse health effects. This is particularly true when preparing
856 solutions from concentrated acid, and for applicators using the vaporizer method, which requires the use of
857 respirator, goggles, and gloves.

858
859 **Evaluation Question #11: Describe all natural (non-synthetic) substances or products which may be**
860 **used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed**
861 **substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).**

862
863 A central challenge of controlling mites on honey bees is that both organisms may be susceptible to the
864 same substances due to biological similarities (Pettis, Rose and Chaimanee 2017). Effective controls need to
865 be more toxic to varroa mites than honey bees so that dosages can be tuned for therapeutic effect. Further
866 complicating matters, the effectiveness of many substances (including OA) is variable, depending on
867 climatic, seasonal, and other factors. For example, Higes et al. (1999) reported OA efficacy against mites as
868 being 73 percent in spring and 94 percent in autumn. Gregorc and Planinc (2001) reported low efficacy of
869 only 44 percent for OA trickled on brood, but 99 percent efficacy for OA trickled on broodless colonies. In
870 general, OA has been found to have high efficacy, commonly between 75-99 percent, when applied during
871 broodless periods (Nanetti 1999; Charrière and Imdorf 2002; Gregorc and Poklucar 2003; Higes, Martín-
872 Hernández and Meana 2006; Bacandritsos, et al. 2007; Martín-Hernández, et al. 2007; Al Toufailya, Scandian
873 and Ratnieks 2015; Adjlane, Tarek and Haddad 2016; Gregorc, Alburaki, et al. 2017).

874
875 In addition to OA, nine active ingredients have been approved by the EPA for use against varroa mites
876 (National Pesticide Information Center 2018b). Several of these may be permitted as nonsynthetic
877 alternatives. Two of these substances are synthetic and permitted for use in organic apiculture in
878 accordance with 205.603(b).

879
880 The brand-name products identified below could potentially be allowed in organic livestock production
881 because they contain an active ingredient that may comply with the NOP National List. However, these
882 products have not been confirmed to meet all other applicable NOP requirements for use as inputs, such as
883 verifying that nonsynthetic ingredients are confirmed as nonsynthetic under NOP Guidance 5033, and that
884 inert ingredients comply with 7 CFR 205.603(e). None of the brand-name products identified below are
885 currently listed as allowed inputs by OMRI or WSDA for use in organic livestock production.

886
887 Nonsynthetic alternatives that are EPA-Registered or FIFRA exempt:

- 888
889 • *Thymol and thyme extracts:* Thymol can be produced from both nonsynthetic (e.g., thyme oil) and
890 synthetic sources (O'Neil, et al. 2013). Like OA and formic acid, thymol is a natural constituent of

891 honey. It can be tasted in honey at a fairly low threshold of around 1.1–1.3 mg/kg in honey but
892 dissipates within 3–4 weeks after treatment (Bogdanov, et al. 1999, Bonheví, Coll and Martínez
893 2016). Thymol has been used experimentally for varroa mite control with good success (Bonheví,
894 Coll and Martínez 2016; Imdorf and Bogdanov 1999), outperforming OA in one experiment in
895 Turkey (Emsen, Dodoloğlu and Genç 2010). Rahimi et al. (2017) found that an extract of thyme
896 using ethanol as the solvent was effective in controlling varroa mites and had no adverse effect on
897 honey bees. Thymol may modulate gamma-aminobutyric acid (GABA), a neurotransmitter
898 involved with muscle activation and glandular stimulation (Rahimi, Del and Moradpour 2017).
899

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

- 903 ○ Apiguard (EPA Reg. No. 79671-1), produced by Vita (Europe) Limited, C/O Landis
904 International, P.O. Box 5126, Valdosta, Ga; and
 - 905 ○ ApiLife VAR (EPA Reg. No. 73291-1), produced by Chemicals Laif S.P.A. (Arysta
906 Lifescience America Inc.), Via Dell 'Artigianato, 13, 35010 Vigonza (PD), Italy.
- 907
 - 908 • *Menthol*: Like thymol, menthol can be produced from either nonsynthetic sources (such as
909 peppermint or other mint oils) or synthetic sources (O'Neil, et al. 2013). No literature was found
910 directly discussing menthol's efficacy or mode of action against varroa mites, though it is used in
911 the EPA registered formulation (below) in combination with other substances due to its efficacy
912 against tracheal mites (Imdorf and Bogdanov 1999; Maeda and Sakamoto 2016).
913

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

- 917 ○ Api Life Var (EPA Reg. No. 73291-1), produced by Chemicals Laif S.P.A. (Arysta
918 Lifescience America Inc.), Via Dell 'Artigianato, 13, 35010 Vigonza (PD), Italy.
- 919
 - 920 • *Oil of Eucalyptus*: In a laboratory study, Ghasemi et al. (2011) found that fumigating with an oil
921 extract from *Eucalyptus camaldulensis* was effective at killing 50 percent of mites at a concentration
922 of 1.74 µL/L of air. The same material also caused mortality to 50 percent of bees at a concentration
923 of 3.05 µL/L air.
924

925 Api Life Var, the EPA-registered product mentioned under menthol, contains oil of eucalyptus as
926 an active ingredient in addition to thymol and menthol.
927

- 928 • *Hop beta acids/lupulones (HBA)*: HBA both repels and causes mortality in mites (DeGrandi-Hoffman,
929 et al. 2012). In both laboratory and field studies, HBA increased mite mortality over controls and
930 was found to be more effective than the synthetic acaricide, fluvalinate. HBA in colonies is
931 ephemeral, disappearing in less than 2 weeks; therefore, multiple treatments are needed to
932 effectively control mites. When treating packaged bees in 0.9 kg containers with strips impregnated
933 with HBA, over 90 percent of mites were killed, with no statistically significant increase in honey
934 bee mortality (DeGrandi-Hoffman, et al. 2012). Larger packages treated with 4 strips had elevated
935 bee mortality compared to controls ($2.7 \pm 0.4\%$ for treatment, compared with $0.62 \pm 0.12\%$ for
936 control).
937

938 One EPA-registered product contains hop beta acid resin:

- 939 ○ Hopguard II (EPA Reg No. 83623-2), produced by Betatec Hop Products, a subsidiary of
940 John I. Haas, Inc., 5185 Macarthur Blvd., NW - Suite 300, Washington DC, 20016.
941

942 Nonsynthetic alternatives that are not EPA registered or FIFRA exempt: 943

- 944 • *Other essential oils (other than thymol, menthol, and oil of eucalyptus)*: Also known as “ethereal oils,”
945 essential oils can either attract or repel varroa mites (Kraus, Koeniger and Fuchs 1994). Oils that

946 attract mites included clove, cinnamon, and menthone. Though not attractive enough to be used as
947 a bait, cinnamon oil causes increased mite mortality. In a laboratory experiment, citronella oil was
948 the most repellent oil, but oil of marjoram was more effective in the field. When embedded in comb
949 foundation wax, oil of marjoram decreased the infestation rate of varroa mites by 46–48 percent.
950 The toxicity of many essential oils is only slightly different for mites and bees, making them
951 unsuitable for use to directly kill mites (Kraus, Koeniger and Fuchs 1994).

952
953 Imdorf and Bogdanov (1999) noted that other than thyme, only a few essential oils have been used
954 successfully as acaricides in field applications: wintergreen oil in combination with a thermal
955 treatment; aerosol spray of thyme-sage oil mixture; and passive evaporation of thymol, oregano oil,
956 and marjoram oil in combination with diluted formic acid. Other essential oils toxic to mites, but
957 not to honey bees at similar concentrations, include lavender, lavandin, and laurel oils (Damiani, et
958 al. 2009).

959
960 Numerous essential oils are FIFRA-exempt as active ingredients, including cinnamon oil, clove oil,
961 and peppermint oil. However, the more efficacious oils noted above (except for thyme oil) are not
962 exempt when used as an active ingredient in a pesticide.

- 963
964 • *Neem oil*: One study demonstrated that when sprayed six times at four-day intervals, neem oil
965 killed 50–80 percent of mites, but also reduced the honey bee brood population by 50 percent and
966 caused up to 50 percent losses of honey bee queens (Melathopoulos, et al. 2000). Neem seed extracts
967 can disrupt hormones related to honey bee caste determination (i.e., what role the honey bee will
968 play within the colony) and cause larval deformities and death within 48 hours of application
969 (Rembold, et al. 1980). Neem oil is a contact insecticide composed of numerous chemical
970 compounds including azadirachtin. While little is known about its mode of action, azadirachtin
971 may stimulate cells involved with feeding inhibition, weakening, and killing insects (Campos, et al.
972 2016).

973
974 There are currently no EPA-registered neem oil formulations for use on honey bees to control
975 varroa mites (National Pesticide Information Center 2018b), and neem oil is not noted as a FIFRA-
976 exempt active ingredient in pesticide formulations (EPA 2015b).

- 977
978 • *Rotenone*: Rotenone is a nonsynthetic botanical pesticide (isoflavonoid) made from plants in the
979 Fabaceae family (legumes), such as *Derris* spp., *Lonchocarpus* spp., and *Tephrosia* spp. It is acutely
980 toxic to insects and fish species, blocking the process of cellular respiration within mitochondria
981 (EPA 2007). In one study, rotenone caused an average mite mortality of 27 percent, which was as
982 effective as OA (with an average mite mortality of 20.85 percent) for controlling varroa mites when
983 brood was present (Gregorc and Poklukar 2003). Its effect on bees was not reported.

984
985 Rotenone is currently only registered with the EPA as a piscicide, or fish pesticide (National
986 Pesticide Information Center 2018a).

- 987
988 • *Nonsynthetic organic acids (acetic, citric, costic, and lactic)*: Nonsynthetic organic acids such as acetic,
989 citric, and costic acid have been compared against OA for efficacy against varroa mites. While citric
990 acid is often produced through fermentation (Verhoff 1985), modern methods of acetic acid
991 production are typically synthetic except for fermentation processes used for table vinegar (Aguiló,
992 Hobbs and Zey 1985). Higes et al. (2006) found that acetic acid and citric acid were no more
993 effective for mites than control groups but increased bee mortality. Sofou et al. (2017) found in field
994 tests that costic acid extracted from the plant *Dittichia viscosa* was either as effective, or 80 percent as
995 effective, as OA (depending on the field) and did not cause bee mortality.

996
997 Acetic, citric, and costic acid are not noted as FIFRA-exempt active ingredients in pesticide
998 formulations (EPA 2015b).

999
1000 Synthetic alternatives that are EPA Registered and on NOP National list:

1001
1002 • *Formic acid* (CAS# 64-18-6): Formic acid is listed on the National List at 7 CFR 205.603(b)(2) with the
1003 following annotation: “for use as a pesticide solely within honeybee hives.” Like OA and thymol, it
1004 is a natural constituent of honey (Bogdanov, et al. 1999). While formic acid occurs naturally,
1005 commercial forms are from synthetic sources (Hietala, et al. 2016). Unlike OA, formic acid is
1006 effective against both phoretic and reproductive mites within sealed brood cells (Rosenkranz,
1007 Aumeier and Ziegelmann 2010), but its effectiveness is variable and dependent on ambient
1008 temperature, and under some conditions (such as when formic acid evaporates too rapidly from
1009 application pads) it can harm bees (Elzen, Westervelt and Lucas 2004). In Florida, Elzen et al. found
1010 that formic acid applied on a saturated pad reduced varroa mites by 39.7 percent. In Argentina,
1011 Eguaras et al. (2001) found that formic acid in a gel matrix reduced varroa mites by 92 percent.
1012

1013 There are three EPA-registered products containing formic acid:

- 1014 ○ For-Mite (EPA Reg No. 61671-3), produced by Mann Lake Ltd. (Landis International, Inc.),
1015 501 S. 1st Street, Hackensack, Mn 56452-2001
- 1016 ○ Mite Away Quick Strips (EPA Reg No. 75710-2), NOD Apiary Products USA Inc., 8345 NW
1017 66th Street #8418, Miami, FL
- 1018 ○ Formic Pro (EPA Reg No. 75710-3)
1019

1020 • *Sucrose octanoate esters* (CAS#s 42922-74-7; and 58064-47-4): Sucrose octanoate is listed on the
1021 National List at 7 CFR 205.603(b)(8) and 205.601(e)(10)⁵ with the following annotation: “in
1022 accordance with approved labelling.” Originally discovered in wild tobacco, sucrose octanoate is
1023 damaging to soft-bodied insects and mites in a similar manner as insecticidal soaps (i.e., it causes
1024 suffocation or disruption of cuticular waxes) (Isman 2006). Against varroa mites, Sammataro et al.
1025 (2008) found that applying a spray of sucrose octanoate esters was no more effective than a non-
1026 treatment control.
1027

1028 No active EPA registrations exist; however, the following product containing sucrose octanoate
1029 was formerly registered:

- 1030 ○ Avachem Sucrose Octanoate [40.0%] (EPA Reg No. 75197-1), produced by Applied Power
1031 Concepts, Inc., 411 E. Julianna Street, Anaheim, Ca 92801-2515.
1032

1033 *OA used in rotation with other products*

1034 The scientific literature does not generally provide prescriptive instructions for an overall varroa mite
1035 management plan. The effectiveness of chemical and biotechnical controls is variable (as an example, see
1036 *Action of the Substance: Variables* for the variability in effectiveness for just OA). Governmental, academic,
1037 and beekeeper interest groups provide pest management manuals that describe general methods and
1038 chemicals for mite control, but do not typically provide specific rotation instructions with efficacy data
1039 supporting or comparing different management plans.

1040 A variety of sources recommend using multiple measures (both chemical and biotechnical) to control
1041 varroa mites on honey bees, including the use of miticides in rotation (Charrière and Imdorf 1999; ATTTA
1042 2017; Ritter 1999; Kristiansen 1999; Rosenkranz 1999; Caron 2015; Mullen 2016; DEFRA 2017). Using
1043 treatments with different modes of action can help prevent mite resistance and increase treatment efficacy
1044 (Rosenkranz, Aumeier and Ziegelmann 2010). None of the literature reviewed suggested OA treatments be
1045 used exclusively, and sources repeated similar, though not identical, overall management schemes.

1046 OA treatments are best suited to broodless conditions (see *Action of the Substance*). If chemical and
1047 biotechnical control strategies are used, different ones are needed throughout all honey bee/colony life
1048 stages. Therefore, if chemical control is part of the varroa management plan, multiple chemicals may be
1049 beneficial. Ritter (1999) divided varroa control into three phases:

1050 1) application of biotechnical (non-chemical) controls at the beginning of the season;

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.

- 1051 2) monitoring and chemical treatments when necessary during the summer; and
1052 3) verification of treatment success and application of additional controls as necessary (of all types)
1053 in the late season.

1054 Similar to Ritter, Kristiansen (1999) recommends drone brood removal in spring (where reproductive mites
1055 concentrate), followed by evaluation of mites in June, application of formic acid after honey harvest, and
1056 finally applying lactic acid or oxalic acid as necessary. Charrière and Imdorf (1999) stress the importance of
1057 mite reduction in August and September using formic acid or thymol, followed by OA (or coumaphos) in
1058 November. According to Ritter, the complete treatment concept applied is more important than the specific
1059 chemicals used.

1060
1061 **Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned**
1062 **substance unnecessary (7 U.S.C. § 6518 (m) (6)).**
1063

1064 Five general approaches are used for non-chemical control of varroa mites: developing resistant honey bee
1065 varieties, using screens at the bottom of colonies to trap and sequester mites away from honey bees, dusting
1066 bees to knock down the population of phoretic mites, removing brood, and heat treatments. These methods
1067 are often complicated, time-consuming, and dependent on proper timing (DEFRA 2017) and may not be
1068 suitable for all operations.

1069
1070 *Resistant/tolerant varieties*

1071 The varroa mite's natural host is the Asian hive bee (*Apis cerana*). This bee is both mite tolerant (i.e., bees
1072 reduce the harmful effects of the mites) and mite resistant (i.e., bees reduce the reproductive success of the
1073 mites). The bees control mites by killing phoretic individuals and removing mite-infested bee pupae from
1074 the colony. Mites are limited to reproducing in drone⁶ brood, leading to a stable host-parasite relationship
1075 (Locke 2016). Similar hygienic behaviors can be produced in Western honey bees (*Apis mellifera*) through
1076 selective breeding, but selection pressure must be maintained continually (Erickson, Atmowidjojo and
1077 Hines 1998). Selection pressure includes "requeening" colonies with varroa-resistant queens and
1078 eliminating susceptible colonies while mating queens only with drones from other varroa-resistant colonies.
1079 Beekeepers do not always adopt such stocks due to historical preferences for specific varieties, or valuing
1080 production or bee gentleness over varroa mite resistance (APHIS 2014). Additionally, because some
1081 beekeepers transport bees from one region to another, one stock of bees may not have the characteristics
1082 necessary for all aspects of their operation. Western honey bees that have been bred for mite resistance
1083 often form smaller colonies and have lower honey production (Locke 2016).

1084
1085 Three active defense traits by bees that support varroa resistance are:

- 1086 • mite non-reproduction (suppression of, or otherwise reducing mite fecundity);
1087 • removal of mite-infested brood by workers; and
1088 • varroa sensitive hygienic behaviors (VSH), whereby bees remove mites when grooming, sometimes
1089 resulting in mites with chewed appendages (APHIS 2014, Locke 2016, Rinderer, et al. 2010).

1090
1091 While many "survivor bees"⁷ are not as productive from a beekeeper's perspective, resistant strains have
1092 been developed – including Russian honey bees, Minnesota Hygienic honey bees, and those from Le
1093 Rucher D'Oc – which have been used successfully on bee operations (APHIS 2014). In some cases, these
1094 strains have allowed beekeeping operations to forego miticides for more than ten years. Purdue University
1095 developed honey bee populations over the course of six years that increased mite chewing behavior during
1096 grooming from 3 percent to 44 percent (APHIS 2014). In Sweden, honey bees artificially infested with
1097 varroa mites on the island of Gotland were left to evolve on their own. After three years, over 80 percent of
1098 the colonies died, but subsequently the mite infestation rate and the winter honey bee mortality rate
1099 decreased. These bees developed a characteristic which reduced the mite population growth rate by
1100 82 percent (APHIS 2014, Locke 2016). Based on a review of genetic studies, Locke speculated that this
1101 characteristic could be a volatile substance produced by bees that influences mite egg production.

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.

1102
1103 Russian, Minnesota Hygienic, and VSH bees are commercially available (Foley's Russian Bees 2018, B and B
1104 Honey Farms, LLC 2018, Wildflower Meadows, Inc. 2018).

1105
1106 *Floor screens*

1107 Adding screens that allow mites, but not bees, to pass through can be used to delay colony infestations
1108 (APHIS 2014). Harbo and Harris (2004) found that hives with an open screen floor had fewer mites
1109 (1.4 mites/bee vs. 2.5 mites/bee), 13–21 percent lower proportion of mites within brood cells, and 10–
1110 17 percent more capped brood, without negative effects on bees. The screens appeared to increase the
1111 amount of time between when mites left one brood cell and entered a new one (9.4 days vs. 4.4 days).
1112 Delaplane et al. (2005) found similar results for screened floors but also observed a 31 percent reduction of
1113 honey and 30 percent reduction of pollen stores per colony as compared with solid floors.

1114
1115 *Dusting*

1116 Dustings with a variety of materials (e.g., powdered sugar, ground pollen, wheat flour) may adhere to
1117 specialized structures on mites called ambulacra, preventing phoretic mites (those that are outside of brood
1118 cells) from attaching to the surface of honey bees (Fakhimzadeh 2001). Powdered sugar applications may
1119 also stimulate honey bee grooming, which in turn leads to mite fall (Rinkevich, Danka and Healy 2017).

1120
1121 Rinkevich et al. (2017) found that mite mortality from powdered sugar treatments combined with screened
1122 bottom boards was not different from untreated groups throughout the entire season, but mite infestation
1123 was slowed in the months of August and September compared with controls. Fakhimzadeh (2001) found
1124 that under field conditions, mite fall due to powdered sugar treatments was significantly higher than in
1125 control groups. Macedo and Ellis (2002) found similar results but recommended the use of powdered sugar
1126 dusting as a monitoring tool, rather than as a control measure. Aliano and Ellis (2005) developed a process
1127 whereby adult bees were driven from the hive into a detachable box using a bee repellent, and
1128 subsequently powdered sugar was applied. The process removed 76 percent of phoretic mites from the
1129 honey bees.

1130
1131 *Brood removal*

1132 Mites preferentially infest drone brood over worker brood (Aliano 2008; Rinderer, et al. 2010). Infested
1133 drone brood combs can be removed without negative effects on colony size or honey production, and this
1134 can reduce final mite population by 50-70 percent (Rosenkranz, Aumeier and Ziegelmann 2010).
1135 Alternatively, brood “traps” can be employed; in a broodless colony, a frame of open (unsealed) brood cells
1136 is added. Mites move to the brood cells to reproduce. These cells are then capped, and the brood combs
1137 with the mites are removed, potentially resulting in more than 90 percent mite reduction (DEFRA 2017; de
1138 Ruijter 1999). Brood traps can also be combined with artificial swarms: The queen is housed/caged in a new
1139 hive that is still in the old location, with the old hive moved some distance away. As worker bees from the
1140 old hive return from foraging, they move to the new hive. After three weeks, all brood in the old hive are
1141 hatched, and brood traps are placed (DEFRA 2017). This process allows the beekeeper to simultaneously
1142 control the brood cycle (and therefore increase the effectiveness of the brood traps) and honey bee
1143 swarming behavior.

1144
1145 *Heat treatment*

1146 Heat treatment, or thermotherapy, requires specialized hive structures such as Linhart’s “Thermosolar
1147 Hive”⁸ and “Be Climatized Hive-Solar”⁹ or heaters such as “The Victor-For Varroa Mite Thermal
1148 Treatment.”¹⁰ The treatment is made possible by the difference in heat tolerance between varroa mites and
1149 honey bees. Varroa mites prefer temperatures between 26–33°C (Rosenkranz, Aumeier and Ziegelmann
1150 2010). Over 37–38°C and 40–70 percent relative humidity (RH), varroa mite female fecundity is no longer
1151 observable, while the same conditions do not affect bee mortality (Le Conte, Arnold and Desenfant 1990). In
1152 brood cells at 40 percent RH and 38°C, varroa mite mortality reaches 100 percent after 24 hours. In one
1153 study, heat treating capped brood for 150 minutes at temperatures between 40–47°C resulted in nearly

⁸ Thermosolar Hive, <http://thermosolarhive.com/en/homepage/>

⁹ Bee Ethic, <https://www.beeethic.com/>

¹⁰ Green Beehives, <http://www.greenbeehives.com/thevictor.html>

1154 100 percent mite mortality, with no observable effects on bees or comb wax (Bičik, Vagera and Sádovská
1155 2016). Similarly, a study conducted on caged and heat treated adult bees demonstrated that after 48h and
1156 40°C, all mites had fallen off their host (J. R. Harbo 2000). Harbo noted that heat treatment is risky as a
1157 technique, because the therapeutic temperatures are close to those that are damaging to bees.
1158
1159

Report Authorship

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1172 All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11 – Preventing
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1174
1175

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