Ozone

Crops

Identification of Petitioned Substance

Chemical Names:
Ozone
Triatromic oxygen

Other Name:
Ozon

Trade Names:
N/A

CAS Numbers:
10028-15-6

Other Codes:
EC No. 233-069-2
ICSC No. 0068
RTECS No. RS8225000
UNII No. 66H7ZZK23N

Summary of Petitioned Use

Ozone is used in organic crop production as a cleaner for irrigation systems. The United States Department of Agriculture (USDA) National Organic Program (NOP) has approved ozone as a “synthetic substance allowed for use in organic crop production” with the stipulation that it is “for use as an irrigation system cleaner only” in 7 CFR 205.601. The NOP has also approved ozone as an allowed synthetic substance “in or on processed products labeled as ‘organic’ or ‘made with organic’” in 7 CFR 205.605.

This technical report was requested by the National Organic Standards Board (NOSB) Crops Subcommittee in support of its sunset review of ozone use in crop production.

Characterization of Petitioned Substance

Composition of the Substance:
Ozone is the minor allotrope of oxygen with three oxygen atoms and a chemical formula of O_3 (Silberberg 2003). Ozone can be represented in two major resonance structures, shown below in Figure 1a (Silberberg 2003, Atkins et al. 2008, Baird and Cann 2008). The two resonance structures exist simultaneously; combining these two forms produces the resonance hybrid structure for ozone (Figure 1b), which most effectively describes the compound. The resonance hybrid structure for ozone is represented by the bent structure of the molecule with the central oxygen atom connected to the outer oxygen atoms by a single bond and partial double bond (Silberberg 2003, Atkins et al. 2008, Baird and Cann 2008).

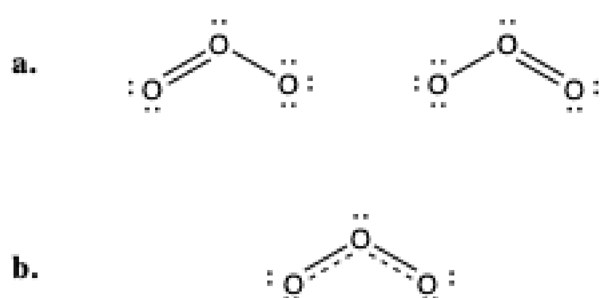

Figure 1

Source or Origin of the Substance:
Ozone is a naturally occurring gas. Most natural ozone is in the stratosphere and makes up the ozone layer. Stratospheric ozone protects the earth and lower atmosphere from high-energy radiation (UV-B, 280 – 320

nm radiation), which is absorbed by the ozone layer (Flommenbaum et al. 2002, Silberberg 2003, Atkins et al. 2008, Baird and Cann 2008). Stratospheric ozone is produced when diatomic oxygen undergoes photochemical dissociation to produce reactive oxygen atoms, as shown below in Equation 1 (Smith 2002, Silberberg 2003, Baird and Cann 2008). The oxygen atoms quickly react with nearby diatomic oxygen molecules in a second step to form ozone, as shown below in Equation 2 (Smith 2002, Silberberg 2003, Biard and Cann 2008). The high-energy UV radiation required to dissociate molecular oxygen—and form oxygen atoms as a reactive intermediate—results in the location of the ozone layer in the stratosphere, where the required radiation is more prevalent (Baird and Cann 2008).

$$O_{2\,(g)} + h\nu \rightarrow 2\,O_{(g)}$$

Equation 1

$$O_{(g)} + O_{2\,(g)} \rightarrow O_{3\,(g)} + \text{heat}$$

Equation 2

Ozone is also found in the troposphere as an important chemical component of smog. Ozone in smog can be produced by many reactions facilitated by nitric oxide (NO) and volatile organic compounds (VOCs), both of which are produced by incomplete combustion reactions (Baird and Cann 2008). Nitric oxide and VOCs can combine with diatomic oxygen, undergoing photochemical reactions that produce smog as a mixture of ozone (O_3), nitric acid (HNO_3), and a variety of organic compounds, as shown below in Equation 3.

$$NO_{(g)} + VOCs_{(g)} + O_{2\,(g)} + h\nu \rightrightarrows O_{3\,(g)} + HNO_{3\,(g)} + \text{organic compounds}$$

Equation 3

Ozone used for agricultural, purification, and industrial applications is synthetic and produced on demand by ozone generators. Most ozone generators apply high voltages to pure oxygen (O_2) or air (gas mixtures of largely N_2 and O_2) (EPA 1999, Smith 2002, USDA 2002, Atkins et al. 2008, Msayleb et al. 2013).

<u>Properties of the Substance:</u>

Ozone is a highly reactive gas. It is one of the most powerful known oxidants, with a reduction potential of +2.08 V (EPA 1999, Smith 2002, Atkins et al. 2008). The oxidizing power of ozone makes it reactive with many organic and inorganic compounds, cell wall and membrane structures, and nucleic and amino acids (Carney et al. 1973, EPA 1999, Flommenbaum et al. 2002, USDA 2002). The bent structure of the molecule makes it a polar compound, which increases its water solubility compared to diatomic oxygen (O_2) (EPA 1999, Silberberg 2003, Biard and Cann 2008). General properties of ozone are listed below in Table 1.

Table 1. Properties of ozone

Property	Ozone
Molecular formula	O_3
Molecular weight	47.998 g/mol
CAS No.	10028-15-6, 10029-15-6
Appearance	Colorless to blue gas
Odor	Pungent chlorine odor
Water solubility	570 mg/L at 20 °C
Melting point	-193 °C
Boiling point	-112 °C
Relative density	1.6 (air = 1)
Reactivity	Strong oxidizer. Unstable and decomposes at ambient temperatures.

Sources: PC 24823, EPA 1999, NJ DOH 2003, Atkins et al. 2008, OT 2014.

Specific Uses of the Substance:

Ozone is used in many applications including as a disinfectant and soil fumigant in agriculture and food processing, as a reactive agent for soil remediation, a primary disinfectant in water treatment and purification, and other industrial applications including as a bleaching agent in textiles and paper production (Carney et al. 1973, EPA 1999, Smith 2002, USDA 2002, NJ DOH 2003, O'Mahoney et al. 2006, Abaidoo et al. 2010, Msayleb et al. 2013, Msayleb 2014, Wang et al. 2014, de Ávila et al. 2017, Pandiselvam et al. 2020). The applications pertaining to its use in agriculture are discussed in greater detail below.

Irrigation system disinfectant/cleaner

Water sources and irrigation systems may contain bacterial, viral, or protozoan pathogens, which can be introduced into the agro-ecosystem and transferred to crops (Abaidoo et al. 2010, Msayleb 2014). The strongly oxidizing nature of ozone acts as a disinfectant for both water sources and irrigation systems to prevent the transfer of pathogens to the agro-ecosystem (EPA 1999, Qiu et al. 2009, Abaidoo et al. 2010).

Ozone can react with organic and inorganic matter. When reacting with organic matter, ozone promotes ozonolysis reactions, which break large, insoluble organic compounds into smaller compounds with higher water solubility (EPA 1999, Smith 2002, USDA 2002, Atkins et al. 2008). Ozonolysis reactions within irrigation systems and agricultural equipment prevent buildup of organic compounds and prevent clogging of irrigation systems (USDA 2002).

Soil fumigant

Ozone is used as a soil fumigant in conventional agriculture to kill weeds and soil-based pathogens prior to planting crops (USDA 2001, USDA 2002, CEC 2002, Sopher et al. 2002, Msayleb and Ibrahim 2011, Msayleb et al. 2013, Msayleb 2014). The reactive nature of ozone reduces the viability of existing weeds by disrupting photosynthesis and plant growth (USDA 2001, Wang et al. 2021). Ozone has broad spectrum efficacy against bacteria, virus, fungi, nematodes, and protozoa pathogens (EPA 1999, CEC 2002, Smith 2002, Sopher et al. 2002, Qiu et al. 2009, Abaidoo et al. 2010, Msayleb and Ibrahim 2011, Msayleb et al. 2013, Msayleb 2014, Pandiselvam et al. 2020). The reduction of soil-based pathogens and reduced viability of weeds with pre-planting ozone soil fumigation has been reported to increased crop yields (Sopher et al. 2002, Msayleb 2014).

Soil remediation agent

Ozone is effective for soil remediation of organic compound contaminants including pesticides, herbicides, and contaminants from refining processes (O'Mahoney et al. 2006, Qiu et al. 2009, Wang et al. 2014, Liu 2018). Ozone reacts with many organic compounds, breaking them apart into more reactive forms and hastening natural biodegradation processes (EPA 1999, USDA 2002, O'Mahoney et al. 2006, Derudi et al. 2007, Wang et al. 2014, Liu 2018).

Approved Legal Uses of the Substance:

The USDA NOP has approved ozone as a "synthetic substance allowed for use in organic crop production" with the stipulation that it is "for use as an irrigation system cleaner only" in 7 CFR 205.601. The NOP has also approved ozone as an allowed synthetic substance "in or on processed products labeled as 'organic' or 'made with organic'" in 7 CFR 205.605.

The United States Food and Drug Administration (FDA) lists ozone as a substance that is generally recognized as safe (GRAS) when used to treat food as an "antimicrobial agent" for bottled water. The FDA specifies a "maximum residual level at the time of bottling of 0.4 milligrams of ozone per liter of bottled water" in 21 CFR 184.1563. When used for water processing and bottling of drinking water, "0.1 parts per million ozone" must be applied to the "water solution in an enclosed system for at least 5 minutes," as stipulated in 21 CFR 129.80. The FDA has approved the use of ozone "as an antimicrobial agent…in the treatment, storage, and processing of foods, including meat and poultry" in 21 CFR 173.368.

The United States Environmental Protection Agency (EPA) approved the use of ozone as a disinfectant of *Cryptosporidium* in public water systems in 40 CFR 141. The EPA has set the "primary and secondary ambient air quality standards for ozone…[at] 0.070 parts per million (ppm)" as a daily maximum 8-hour average in 40 CFR 50.19.

Action of the Substance:

Irrigation system disinfectant/cleaner

The disinfection potential of ozone is due to its strongly oxidizing character. Ozone treatments result in the oxidation of cellular walls and membranes of bacteria and protozoa (USDA 2002, Msayleb 2014). Ozone may also be transported into the cell, where it facilitates the oxidation of nucleic acids, specifically purines and pyrimidines (EPA 1999, USDA 2002). Once inside the cell, ozone may disrupt proper function of organelles, including the mitochondria, and interfere with DNA sequencing (Flommenbaum et al. 2002, Msayleb et al. 2013). Ozone inactivates viruses through reactions with viral capsids, which may be completely eroded via oxidative reactions. Deterioration of capsid proteins prevents viruses from attaching to host cells and may also rupture the virus and spill its nucleic material (EPA 1999).

Ozone reacts with organic compounds found within water systems, which are oxidized to smaller, more water-soluble compounds (EPA 1999, USDA 2002). A prominent oxidation reaction is ozonlysis, which cleaves carbon–carbon double bonds (Atkins et al. 2008). Organic compounds oxidized with ozone are transformed into a variety of more water-soluble compounds, including aldehydes and carboxylic, aldo, and ketoacids (EPA 1999, USDA 2002). In addition to breaking down large organic compounds within water systems, the smaller compounds produced via these oxidative processes are more biodegradable, which facilitates their removal from the irrigation system.

The efficacy of ozone as an oxidant is dependent on environmental conditions (Msayleb and Ibrahim 2011, Msayleb 2014). The stability of ozone decreases at increased temperatures (EPA 1999, Qiu et al. 2009, Msayleb and Ibrahim 2011, Msayleb 2014). Additionally, Henry's Law states that gas solubility is inversely related to temperature (Silberberg 2003). Therefore, low temperature application of ozone improves its lifetime in aqueous environments and its efficacy as a disinfectant (Msayleb and Ibrahim 2011, Msayleb 2014).

In addition to the direct oxidation of pathogens and organic compounds, ozone reacts with water and decomposes to form the even more powerful oxidant, hydroxyl radical (OH), as shown below in Equation 4 (EPA 1999, Baird and Cann 2008). Hydroxyl radicals are among the few oxidants stronger than ozone, with a reduction potential of +2.8 V (EPA 1999). Due to the oxidation strength of hydroxyl radicals, they react indiscriminately with any substance capable of being oxidized, giving them lifetimes on the order of microseconds (EPA 1999). Due to the high reactivity of hydroxyl radicals, they are not formed in large concentrations (their estimated maximum concentration is 10^{-12} M) and react almost immediately upon formation (EPA 1999).

$$O_{3\,(aq)} + H_2O_{(l)} \rightarrow O_{2\,(g)} + 2\ OH_{(aq)}$$

Equation 4

Hydroxyl radical has reduced efficacy as a disinfectant of pathogens due to its increased reactivity, which reduces the selectivity of its oxidation processes (EPA 1999). The higher reduction potential of hydroxyl radical makes it more effective for the oxidation of organic matter, and it can oxidize a wider range of organic compounds compared to ozone (EPA 1999, Liu et al. 2018). The increased strength of the oxidant makes it especially effective against aromatic compounds, which are resistant to oxidative processes due to their thermodynamic stability (Liu et al. 2018).

Environmental conditions dictate whether oxidation occurs primarily through direct ozone oxidation, or oxidation from the secondary hydroxyl radical. Ozone is the predominant oxidant under acidic conditions, due to the decreased stability of hydroxyl radicals, which are quickly neutralized by acidic substances (EPA 1999). Basic conditions facilitate the decomposition of ozone to form hydroxyl radicals, which are also stabilized at high pHs. The more rapid formation and increased stability of hydroxyl radicals at high pHs make them the predominant oxidant under basic conditions (EPA 1999).

Soil fumigant

Ozone is used as a soil fumigant in conventional agriculture to kill weeds and soil-based pathogens prior to planting crops (USDA 2001, USDA 2002). The reactive nature of ozone reduces the viability of existing weeds by disrupting photosynthesis and plant growth (Carney et al. 1973, Wang et al. 2021). Ozone in soil systems may also harm root growth by oxidizing root tissue (Myasleb 2014, Wang et al. 2021). Increasing ozone concentrations within the troposphere have been reported to cause oxidation of lipid membranes and waxes in leaves, disrupt photosynthesis, and to stunt biomass growth above and below ground (Carney et al. 1973, Hofmockel et al. 2011, Agathokleous et al. 2020, Wang et al. 2021).

As described above in the mode of action for ozone as an irrigation system disinfectant/cleaner, ozone has broad spectrum efficacy against bacteria, virus, and protozoa pathogens (Qiu et al. 2009, Msayleb and Ibrahim 2011, Msayleb 2014). Additionally, ozone is effective against pathogenic fungi and nematodes found in soil systems (Msayleb and Ibrahim 2011, Msayleb 2014). The inactivation of soil pathogens proceeds through the same mode of action described above in the discussion of ozone as an irrigation system disinfectant/cleaner.

Either ozone or hydroxyl radicals may be the primary oxidant in soil fumigation applications. As described above and illustrated in Equation 4, ozone reacts with water to form hydroxyl radicals. This secondary oxidant is more likely to be formed in wet soils and alkaline soils, while ozone is expected to be the dominant oxidant in acidic soils (Myasleb 2014, Wang et al. 2014).

The efficacy of ozone as a soil fumigant is dependent on the specific environmental conditions of the soil it is applied to (Qiu et al. 2009, Msayleb 2014). Ozone reacts with both organic and inorganic compounds. These reactions make the soil composition an important factor in the efficacy of soil fumigation processes. Soils rich in metals and organic matter will increase ozone demand and reduce the efficacy of soil fumigation for the removal of soil-based pathogens (Qiu et al. 2009, Msayleb and Ibrahim 2011, Msayleb 2014, Wang et al. 2014).

Ozone is more stable and water soluble at low temperatures, which results in the increased efficacy of ozone soil fumigation in winter months prior to spring planting (Msayleb 2014). The literature shows a lack of consistency in the reported role of soil moisture content in the effectiveness of soil fumigation programs (Qiu et al. 2009, Msayleb and Ibrahim 2011). Some studies have reported improved soil fumigation outcomes in dry soils, which allow for a longer lifetime for ozone and enable greater soil penetration (Qiu et al. 2009). Dry soils also inhibit the formation of hydroxyl radical, resulting in the more selective ozone being the primary oxidant (EPA 1999, Qiu et al. 2009). Other studies state the opposite, claiming moist soils are the most effective for ozone fumigation and even recommending irrigation prior to ozone application (Msayleb and Ibrahim 2011). These studies cite the inaccessibility of inorganic and organic soil compounds that may compete with pathogen disinfection for ozone oxidation in wet soil systems (Qiu et al. 2009).

Soil remediation agent

Ozone is effective for soil remediation of organic contaminants, including pesticides, herbicides, and contaminants from refining processes (O'Mahoney et al. 2006, Wang et al. 2014). Oxidation processes may occur through direct oxidation by ozone or through the secondary hydroxyl radical formed in moist and alkaline soils, as discussed above and described in Equation 4 (Wang et al. 2014, Liu et al. 2018). Hydroxyl radical acts as a nonselective oxidant that will rapidly react with surrounding organic matter in the soil.

Whether oxidized by ozone or hydroxyl radical, ozone-based soil remediation converts toxic, recalcitrant substances such as polycyclic aromatic hydrocarbons (PAHs) into carboxylated and/or hydroxylated benzenes and quinones (O'Mahoney et al. 2006, Derudi et al. 2007, Liu et al. 2018). The products of ozone oxidation are considered to be less harmful than the initial organic contaminants, and more readily undergo biodegradation by soil microorganisms (EPA 1999, O'Mahoney et al. 2006, Liu et al. 2018).

Like with soil fumigation applications, the efficacy of ozone remediation is dependent on the soil conditions. As discussed above, soils with high concentrations of oxidizable organic and inorganic matter will increase ozone demand (O'Mahoney et al. 2006, Wang et al. 2014). The effectiveness of ozone remediation treatments in relation to soil moisture are dependent on the type of soil and specific contaminant (O'Mahoney et al. 2006, Wang et al. 2014). Soils that are low in oxidizable compounds are more effective in dry soil than moist soil. This is due to reduced accessibility of contaminants in moist soils, whose surfaces are covered with water and which facilitate the conversion of ozone to the non-selective hydroxyl radical (Equation 4) (O'Mahoney et al. 2006). However, soils that are high in oxidizable compounds provide more effective remediation when moist, since this reduces accessibility of metal oxides within the soil that catalyze ozone degradation. Additionally, the application of ozone to moist soils is more effective against compounds that resist oxidation, including PAHs, since increased water content increases the concentration of the more potent hydroxyl radical oxidant (Wang et al. 2014).

Combinations of the Substance:

When used for disinfection, ozone may be combined with metal oxides or hydrogen peroxide. Metal oxides, specifically MnO_2, Al_2O_3, and Fe_2O_3, have been reported to catalyze the formation of reactive hydroxyl radical due to their unique surfaces (Wang et al. 2014). Hydrogen peroxide also facilitates the conversion of ozone in aqueous environments to hydroxyl radicals, increasing the oxidizing power of aqueous ozone solutions (EPA 1999). The conversion of aqueous ozone to hydroxyl radical also increases the effective solubility of ozone by linking the dissolution process to the formation of hydroxyl radical (Equation 4), which constantly pulls additional gaseous ozone into the solution to replace the ozone lost to radical formation (Msayleb 2014).

Status

Historic Use:

Ozone has a long history of use as a disinfectant. Ozone was first used in 1893 as a disinfectant water treatment in the Netherlands (EPA 1999, Sopher et al. 2002, USDA 2002). While ozone is long established as a disinfectant in Europe, this use is relatively new in the United States. The NOSB recommended that ozone be added to the National List for use as a disinfectant in food processing in 1995, and it was subsequently added to the National List (NOSB 1995b). The United States National Organic Program (NOP) further added ozone to the National List "for use as an irrigation system cleaner only" in 2003 (USDA 2003).

Organic Foods Production Act, USDA Final Rule:

Ozone is not listed in the Organic Foods Production Act (OFPA) of 1990. Ozone is listed as an allowed synthetic substance "in or on processed products labeled as 'organic' or 'made with organic'" in 7 CFR 205.605. Ozone is listed as a "synthetic substance allowed for use in organic crop production… for use as an irrigation system cleaner only" in 7 CFR 205.601.

International

Canada - Canadian General Standards Board Permitted Substances List (updated November 2015)

The CAN/CGSB-32.311-2015 lists ozone in Table 6.3 as an "food additive," in Table 6.5 as a "processing aid," and Table 7.3 as a "food-grade cleaner, disinfectant and sanitizer permitted without a mandatory removal event."

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

Ozone is not listed in the CODEX GL 32-1999.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008
Ozone is not listed in EC No. 834/2007 or EC No. 889/2008.

Japan Agricultural Standard (JAS) for Organic Production
Ozone is listed in JAS Notification No. 1605 as a "substance for preparation" for organic plants. Ozone is listed in JAS Notification No. 1606 as a "food additive" for organic processed foods that is "limited to be used for processed foods of plant origin, animal intestine disinfection, or as egg cleansing." Ozone is listed in JAS Notification No. 1608 as a "substance for preparation" for organic livestock products that is "limited to the use for disinfecting meat and poultry at slaughter, or washing eggs."

International Federation of Organic Agriculture Movements (IFOAM)
Ozone is included in The IFOAM Norms for organic production and processing as an "equipment cleanser and equipment disinfectant."

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

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

A) Ozone has been approved for use in organic agriculture as an "irrigation system cleaner," which includes use as a "equipment cleanser" as described in the above question.

B) Ozone is not included on EPA List 4, and it is not included in 40 CFR 180.

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

Due to the reactive and oxidizing character of ozone, it is produced onsite at the point of use (Sands et al. 2002, O'Mahoney et al. 2006, Qiu et al. 2009, Msayleb et al. 2014, de Áliz et al. 2017, Liu 2018, Pandiselvam et al. 2020). The specific location of the ozone equipment and production depends on its use and includes in stationary tanks used to house ozonated water supplies, to be applied to harvested crops via spray lines, or to field crops when transported via tractor (Sands et al. 2002). In cases where the soil or harvested crops are treated with gaseous ozone the substance is produced at the fumigation chamber or soil covering (O'Mahoney et al. 2006, de Ávila et al. 2017, Pandiselvam et al. 2020). In water purification applications, ozone is produced at the point of treatment and is introduced to the treatment stream with a contactor, diffuser, turbine mixers, or injector apparatus (EPA 1999).

Ozone used in agricultural applications is synthetically produced by the application of electrical energy facilitated by ozone generators (EPA 1999, USDA 2002). The reaction sequence within ozone generators is a two-step process that is similar to the stratospheric process. Ozone generators apply electrical energy (*E*) to break apart diatomic oxygen into highly reactive oxygen atoms, as shown below in Equation 5 (EPA 1999, CEC 2002, USDA 2002, Qiu et al. 2009). Like in the stratosphere, the oxygen atoms quickly react with surrounding diatomic oxygen to form ozone and release heat in an exothermic reaction, as shown in Equation 2 in the "Source or Origin of the Substance" section (EPA 1999, USDA 2002, Silberberg 2003, Baird and Cann 2008).

$$O_{2\,(g)} + E \rightarrow 2\,O_{\,(g)}$$

Equation 5

Nearly all ozone for agricultural applications, and most other applications, is produced through the corona discharge method (EPA 1999, Sands et al. 2002, Smith 2002, USDA 2002 O'Mahoney et al. 2006, Qiu et al. 2009, Msayleb et al. 2014, de Áliz et al. 2017, Liu 2018, Pandiselvam et al. 2020). In this method, oxygen gas (either as a pure gas or as an air mixture) is passed through electrodes that are separated by dielectric and discharge gaps (EPA 1999, Smith 2002, Msayleb et al. 2013). The application of electrical potentials to these electrodes results in the passage of electrons across the gaps, which have sufficient energy to facilitate the dissociation of diatomic oxygen to oxygen atoms (Equation 5) (EPA 1999, Smith 2002, USDA 2002).

The production of ozone through corona discharge requires high electrical potentials to generate the oxygen atoms required for ozone formation. This is generally achieved through the application of a large overpotential, making ozone generation an electrically inefficient process where approximately 75% of the applied energy is lost to light and heat (EPA 1999, CEC 2002).

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

As described in the "Source or Origin of the Substance" section, ozone is a naturally occurring compound that exists in the stratosphere, forming the atmospheric ozone layer. Additionally, ozone is created in the troposphere, primarily as a byproduct of pollutants from incomplete combustion, and it is among the primary components of smog. However, when used in agricultural applications, including organic crop production, ozone is chemically created onsite by an ozone generator, as described in Evaluation Question 2. The formation of ozone from diatomic oxygen is facilitated by the application of electrical energy, primarily through corona discharge. The production of ozone from diatomic oxygen by applied voltages results in the classification of ozone as a synthetic substance (NOSB 1995b, USDA 2002).

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

Ozone is a highly reactive substance with a short environmental lifetime (USDA 2002, O'Mahoney et al. 2006, Msayleb 2014). The specific lifetime of ozone in the environment is dependent on temperature, with more rapid decomposition at increased temperatures (Qiu et al. 2009, Msayleb and Ibrahim 2011, Msayleb 2014). The lifetime of ozone and its degradation pathway is also dependent on the phase of the ozone (i.e., gas or aqueous) (EPA 1999). The primary degradation pathway for ozone is decomposition to diatomic oxygen, as shown below in Equation 6, which may occur in both the aqueous and gas phases (O'Mahoney et al. 2006, Baird and Cann 2008, Msayleb 2014). Ozone has a typical lifetime of minutes to days under ambient temperatures in the atmosphere before breaking down to form diatomic oxygen (Baird and Cann 2008, Msayleb 2014). Diatomic oxygen dissipates into the atmosphere, of which it is a major component (~22%), leaving behind no residual environmental contaminant (EPA 1999, CEC 2002, O'Mahoney et al. 2006, Baird and Cann 2008, Msayleb and Ibrahim 2011, Msayleb et al. 2013, Msayleb 2014, Pandiselvam et al. 2020).

$$2\,O_3 \rightarrow 3\,O_2$$

Equation 6

Ozone has a shorter lifetime in aqueous environments, where it can undergo decomposition to diatomic oxygen (Equation 6) or hydroxyl radical (Equation 4) (O'Mahoney et al. 2006, Liu et al. 2018). When ozone decomposes to hydroxyl radical in aqueous solutions, the hydroxyl radical quickly oxidizes the surrounding compounds and has an environmental lifetime on the order of microseconds (EPA1999, O'Mahoney et al. 2006, Liu et al. 2018).

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

As described in Evaluation Question 4, ozone and its byproducts have short environmental lifetimes of days or less. As described in the "Action of the Substance" section, both ozone and hydroxyl radical are strong oxidants that react with compounds and organisms within the environment. These oxidants have been reported to inhibit photosynthesis and reduce plant and root growth (Hofmockel et al. 2011, Agathokleous et al. 2020, Wang et al. 2021). While ozone is unlikely to have fatal outcomes in established plants, it does increase their susceptibility to other environmental stressors, including drought, lack of nutrients, and pathogens (Agathokleous et al. 2020).

As described in the "Action of the Substance" section, ozone and hydroxyl radical oxidants are highly reactive and are effective broad-spectrum disinfectants against a variety of organisms, including bacteria, fungi, nematodes, protozoa, and viruses. Ozone is often used for its disinfecting power, for example, it can be used as an irrigation system cleaner and soil fumigant (USDA 2002, Msayleb and Ibrahim 2011, Msayleb et al. 2013, Msayleb 2014). However, the broad-spectrum nature of ozone and hydroxyl radical will also result in sterilization of beneficial soil organisms (USDA 2002, Agathokleous et al. 2020). While soil pathogens have been reported to be more sensitive to oxidation than other soil organisms, the literature shows a reduction in soil biodiversity corresponding with increased atmospheric ozone (USDA 2002, Msayleb 2014, Agathokleous et al. 2020).

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

As described above in Evaluation Questions 3 and 4, ozone and its byproducts have short environmental lifetimes and leave no environmental residues. As described in Evaluation Question 2, the production of ozone by on-site generators is an inefficient process in which approximately 75% of the applied electricity is lost to heat and light. Due to the energy-intensive nature of ozone production, environmental contamination may occur from its manufacture in the form of carbon dioxide emissions if electricity is generated by burning fossil fuels (Silberberg 2003, Baird and Cann 2008).

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

As described in the "Action of the Substance" section, ozone reacts with both organic and inorganic compounds. It reacts with metals, which are converted into oxides and hydroxides (USDA 2002, Msayleb and Ibrahim 2011). Metal oxide and hydroxides typically have low water solubility, which also reduces their bioavailability (USDA 2002, Silberberg 2003, Baird and Cann 2008, Msayleb and Ibrahim 2011). There are a variety of metals that are important micronutrients for plant growth, and the application of ozone may result in micronutrient deficits (USDA 2002, Msayleb and Ibrahim 2011).

Ozone oxidation of organic compounds results in degradation to smaller, more water-soluble compounds. These smaller compounds are also more bioavailable and undergo more facile biodegradation in soil systems (Msayleb and Ibrahim 2011). Additionally, soil remediation studies have reported improvements in soil quality and nutrient availability following the ozone oxidation of large organic compounds, including pyrethroids (e.g., bifenthrin, deltamethrin), phthalimides (e.g., captan), benzimidazoles (e.g., carbendazim), triazoles (e.g., tebuconazole, difenoconazole), aminopyridines (e.g., cyprodinil), and phenols (e.g., pentachlorophenol, *p*-nitrophenol) (USDA 2002, Msayleb and Ibrahim 2011, Wang et al. 2014, de Ávila et al. 2017, Paandiselvam et al. 2020). However, there are a variety of organic compounds that are used in organic crop production as nutrients, pesticides, and animal repellents, all of which may undergo oxidation and be rendered ineffective after exposure to ozone.

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

As described in the "Action of the Substance" section and Evaluation Questions 3–7, ozone can oxidize most substances and has been shown to exhibit toxicity to plants and soil organisms. Ozone disrupts photosynthesis and reduces plant growth, making it harmful to crops (USDA 2002, Hofmockel et al. 2011, Agathokleous et al. 2020, Wang et al. 2021). The oxidation of metals in the soil to oxide and hydroxide forms may further inhibit crop growth by reducing micronutrient availability (USDA 2002, Msayleb and Ibrahim 2011). Ozone is an effective broad-spectrum disinfectant and has been shown to reduce biodiversity of soil microorganisms (Agathokleous et al. 2020).

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

As described in Evaluation Questions 3–8, ozone has a short environmental lifetime and leaves no residual contaminants. However, the reactive nature of ozone makes it a toxic substance capable of oxidizing most compounds. As discussed in the "Source or Origin of the Substance" section, tropospheric ozone is a major component of smog. Additionally, ozone is a more potent greenhouse gas than carbon dioxide, and tropospheric ozone contributes to global warming by trapping heat in the lower atmosphere (Baird and Cann 2008). While stratospheric ozone is beneficial as it forms the ozone layer that protects the Earth from harmful ultraviolet rays, tropospheric ozone is harmful as both an oxidant and greenhouse gas (Baird and Cann 2008). Moreover, ozone is denser than air, making it unlikely to migrate from the troposphere, where it is a harmful substance, to the stratosphere, where it is a beneficial substance.

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

Ozone is a respiratory irritant and may cause chronic lung disease (Flommenbaum et al. 2002, USDA 2002, NJ DOH 2003, EPA 2015). The pulmonary toxicity of ozone is due to its oxidation of lipid membranes, resulting in cell death and inflammatory cascades (Flommenbaum et al. 2002). Repeated exposure to gaseous ozone may result in lung damage and chronic lung conditions (Flommenbaum et al. 2002, NJ DOH 2003). The ability of ozone to oxidize DNA may also result in genetic changes and lung cancer (NJ DOH 2003). Exposure to ozone has also been reported to cause headaches, chest pain, and nausea (USDA 2002, NJ DOH 2003). Due to the hazardous nature of ozone gas, the United States Occupation Health and Safety Administration (OSHA) limits 8-hour ozone exposure to levels of 0.1 ppm in 29 CRR 1910.1000.

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

Chlorine, chlorine dioxide, hypochlorous acid, bleach, organic acids (e.g., acetic acid, citric acid, lactic acid, peracetic acid), hydrogen peroxide, alcohols (e.g., methanol, ethanol), and soaps offer alternatives to ozone for the disinfection/cleaning of irrigation system components. Chlorine, chlorine dioxide, hypochlorous acid, and bleach offer a similar mode of action to ozone for disinfection applications. All these chlorine-containing compounds are strong oxidants that kill bacteria, viruses, and protozoa by oxidation of cellular walls and membranes and also disrupt protein function (USDA 2011, USDA 2015c, USDA 2018). Like ozone, these chlorine-based disinfectants are generally unstable and have short environmental lifetimes (Baird and Cann 2008, USDA 2011, USDA 2015c, USDA 2018). However, these chlorine-containing disinfectants leave chemical residues, including chlorite (ClO_2^-), hypochlorite (ClO^-), chlorate (ClO_3^-), and chloride (Cl^-) ions. Additionally, these chlorine-based disinfectants react with organic compounds to form chloro-organic compounds, which are known to include toxins with long environmental lifetimes (Baird and Cann 2008, USDA 2011, USDA 2015c, USDA 2018). Moreover, these substances are weaker oxidants than ozone (EPA 1999, Baird and Cann 2008).

Peracetic acid and hydrogen peroxide are also disinfectants whose mode of action is based on their oxidizing character. Like ozone, hydroxyl radical, and the chlorine-based disinfectants discussed throughout this report, peracetic acid's and hydrogen peroxide's oxidizing character makes them effective broad-spectrum disinfectants against bacteria, viruses, fungi, and protozoa, as they degrade cellular walls and membranes and disrupt protein function (USDA 2015b, USDA 2016). Peracetic acid and hydrogen peroxide also have short environmental lifetimes; peracetic acid degrades to acetic acid, while hydrogen peroxide degrades to water and diatomic oxygen (USDA 2015b, USDA 2016). While peracetic acid and hydrogen peroxide are more environmentally friendly than the chlorine-based oxidants described above, they are weaker oxidants than both the chlorine-based oxidants and ozone.

Organic acids (e.g., acetic acid, citric acid) also act as disinfectants, but act through non-oxidative pathways. Organic acids kill pathogens through changes to the pH of a solution and/or cytoplasm. (Flommenbaum et al. 2002, USDA 2015d). The acidic nature of these compounds disrupts membrane and protein structure by changing surface charges through reduced pH (Flommenbaum et al. 2002). Organic acids may be transported through cellular membranes, which decreases the pH of the cytoplasm to facilitate membrane degradation and disrupts interior protein structures (USDA 2015d).

Alcohols (e.g., methanol, ethanol) and soaps are additional alternatives to ozone for disinfection and cleaning of irrigation systems and disrupt cellular membranes and protein structure through non-oxidative processes. Both alcohols and soap molecules reduce the polarity of aqueous environments. Alcohols and soaps facilitate hydrophobic interactions, which disrupt the structure of cellular membranes and proteins (NOSB 1995a, Silberberg 2003, USDA 2015e). Additionally, the increased hydrophobic interactions may facilitate the removal of organic compounds from irrigations systems by increasing their solubility compared to water alone (Silberberg 2003, USDA 2015e).

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

Steam treatment provides an alternative to ozone as an irrigation system disinfectant .

Report Authorship

The following individuals were involved in research, data collection, writing, editing, and/or final approval of this report:

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All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11—Preventing Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.

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