UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM **July 9, 2020**

Subject: No reasonable expectation of residue from antimicrobial ozone use in industrial and commercial food processing establishments.

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Purpose

The purpose of this document is to provide a rationale supporting a "no reasonable expectation of residues" for ozone used in industrial (e.g., meat processing plants) and commercial (e.g., restaurants, bars, mobile food service vehicles) food processing establishments as a gas and/or dissolved in water. Based on this finding, there is no need for a Federal Food, Drug, And Cosmetics Act (FFDCA) tolerance exemption for the antimicrobial uses of ozone.

The main points of this document are to demonstrate that:

1. Residues of toxicological concern from the use of ozone are not expected in/on raw agricultural commodities (RACs) or processed commodities after fumigation.
2. Exposure to ozone or its degradation products via the oral route (i.e., – in/on foods) is not supported by the physical/chemical properties of ozone.
3. Accordingly, clearances under FFDCA Section 408 (tolerances or exemptions from the requirement of tolerances) for residues of ozone or its degradation products in this context (directly applied to food or indirectly applied to food contact surfaces) are not necessary.

Introduction and Physico-chemical Properties

Ozone, (O_3), is a triatomic allotrope of oxygen (a form of oxygen in which the molecule contains three atoms instead of two as in the common form) that accounts for the distinctive odor of the air after a thunderstorm or around electrical equipment. Ozone is a highly reactive elemental molecule that is continuously produced in the upper atmosphere by the action of solar ultraviolet radiation on atmospheric oxygen. Ozone is 1.5 times as dense as oxygen; at -112° C (-170° F) it condenses to a dark blue liquid which freezes at -251.4° C (-420° F). The gas decomposes rapidly at temperatures above 100° C (212° F) or, in the presence of certain catalysts, at room temperatures. Although it resembles oxygen in many respects, ozone is much more reactive; hence, it is an extremely powerful oxidizing agent, particularly useful in converting olefins into aldehydes, ketones, or carboxylic acids. It is an antimicrobial agent used as a disinfectant for air and water, the production of bottled water, as well as in the treatment of meat, poultry and other foodstuffs, but is not a registered EPA active ingredient[1]. Because ozone can decolorize many substances, it is used commercially as a bleaching agent for organic compounds as well as to remove objectionable odors and flavors. It has a role as a disinfectant, a tracer, an electrophilic reagent, an oxidizing agent, and an antiseptic drug.

In 2000, the Electric Power Research Institute, Food Technology Alliance, Energy Delivery and Utilization submitted a direct food additive petition (FAP) to the US Food and Drug Administration (FDA) entitled "Ozone as an Antimicrobial Agent for the Treatment, Storage and Processing of Foods in Gas and Aqueous Phases."[2] The purpose of the FAP was to gain formal regulatory approval to use ozone for food processing uses. The petition outlined the current state of the science of ozone use in food processing technology, including but not limited to use sites, use rates, application times, quality control tests, and safety considerations. FDA granted the petition[3] and amended 21 CFR 173.368 to allow petitions for ozone as secondary direct food additives in food for human consumption.

This memorandum relies on the petition's characterization of typical ozone generation technologies, application use rates, and test data regarding the lifetime of ozone directly or indirectly applied to food. Typical use rates for a variety of foodstuffs are included in the petition and can be found in the literature (as cited below). These use rates are used to calculate residue values. With a residue value and a half-life, conservative estimated times to reach no expectation of residue can be calculated.

Ozone is also known as reactive oxygen and has poor stability at room temperature and atmospheric pressure, with a half-life in air and water of 20–50 min. Ozone breaks down/degrades into oxygen molecules and oxygen atoms. Literature studies have shown that the half-life of ozone in peanuts is 3-8[4] minutes and approximately 14 minutes on rice grain[5]. Furthermore, ozone molecules decompose into oxygen without producing any additional traces of degradation byproducts from ozone itself. Therefore, ozone does not cause secondary contamination, and there is no residual gas to remove after use. It is important to note that there may be residual oxidative byproducts from the use of ozone that are not related to the ozone molecule.

Assumptions and Half-Life Calculations

Assumptions:

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[1] 41 Fed. Reg. 51065 1976, specifically identifies ozone generators as devices rather than pesticides.

[2] Electric Power Research Institute, Ozone as an Antimicrobial Agent for the Treatment, Storage and Processing of Foods in Gas and Aqueous Phases, Direct Food Additive Petition filed with FDA, June 2000 (EPRI, 3412 Hillview Ave., Palo Alto, CA 94304).

[3] FDA, Secondary Direct Food Additives Permitted in Food for Human Consumption, Federal Register 2001, 66, 123, 33829-33830.

[4] Alencar, E.; Faroni, L.; Martins, M.; Costa, A.; Cecon, P. Decomposition kinetics of gaseous ozone in peanuts. *Eng. Agric. Jaboticabal.*, **2011**, *31*, 930-939.

[5] Ravi, P.; Venkatachalam, T.; Rajamani, M. Decay rate kinetics of ozone gas in rice grains. *Ozone: Sci. Eng.* **2015**, *37*, 450-455.

Ozone can be applied to food or food contact surfaces in one of three ways: dissolved in water (aqueous form), as a gas (gaseous form), or in combination with another compound to act synergistically (adjunct form). Although use rates vary between commodities, information from industry (which provided 30 different direct and indirect food use sites and associated ozone use rates) generally indicates that the gaseous form tends to be used in higher concentrations (0.6 – 50 ppm) than the aqueous forms (0.12 – 8 ppm). This is understandable because the gaseous form of ozone also has a lower reaction rate than the other forms. The adjunct form is deliberately low (~2 ppm) as its mode of action is to act as an initiator to create more powerful oxidizers rather than to directly react with microbes.

Ozone decays through several mechanisms. Particularly significant mechanisms are temperature-enhanced spontaneous decay, pH-enhanced spontaneous decay, alkalinity-enhanced decay, and interactions with organic matter.

Ozone may be applied to food processing equipment, resulting in indirect exposure to food. The chemistry of ozone is well understood to be highly reactive with surfaces such as metals, metal oxides, carbon fibers, and inert materials.[6] Additionally, concentrations of ozone used on food contact surfaces (0.6 - 1.5 ppm) are typically significantly lower than ozone applied to food. [3,4,7]

The standard default assumption for commercial and industrial indirect exposure of food in the absence of data is that 100% of the applied antimicrobial pesticide residues deposited on equipment are transferred into the food. However, this assumption is not valid for ozone at the concentrations used for food contact surfaces because ozone is expected to decompose within minutes given the inherent instability of the molecule once it contacts the surface material. As a result, negligible ozone residue is expected to be available to transfer from indirect exposure. Direct exposure uses conservative calculations which are expected to overestimate any remaining ozone and be inclusive of negligible ozone residue transfers from indirect exposure.

Below is information regarding residue concentrations of ozone directly applied to food or indirectly applied to food contact surfaces for different forms of ozone (i.e., aqueous ozone and ozone gas).

<u>Half-Life Calculations</u>:

To determine how much ozone is left after applications, Avogadro's Constant (6.022×10^{23}) is used in calculations. This allows calculation of the number of molecules in a given mass for a given substance. Calculations will determine time necessary to reach 1 molecule (the formula used does not allow 0 molecules to be input), with the understanding that the final molecule will degrade immediately afterward. To find the time necessary to reach 1 molecule the following formula is used:

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[6] Batakliev, T.; Georgiev, V.; Anachkov, M.; Rakovsky, S.; Zaikov, G. Ozone Decomposition. *Inter. Tox.* **2014**, *7*, 47-59.

[7] Guzel-Seydim, Z.; Greene, A.; Seydim, A. Use of ozone in the food industry. *Lebensm.-Wiss. u-Tech.* **2004**, *37*, 453-460.

$$\frac{t_{1/2} log\left(\frac{N(t)}{N_0}\right)}{log\left(\frac{1}{2}\right)} = t \qquad \text{(Eq. 1)}$$

Where N(t) is the quantity of the substance, N_0 is the initial quantity of the substance, t is the time elapsed, and $t_{1/2}$ is the half-life of the substance.

For the purposes of this document, Equation 1 is calculated as follows:

$$\frac{(t_{1/2}\ hr)\ log\left(\frac{[initial\ quantity\ ozone]\ g \times \frac{1 mol}{48 g} \times \frac{6.022\times10^{23} molecules\ ozone}{mol}}{1\ molecule\ ozone}\right)}{log\left(\frac{1}{2}\right)} = time\ (hr) \qquad \text{(Eq. 2)}$$

Form: Gaseous Ozone

Gaseous ozone half-life data are less common than aqueous ozone. However, the available data indicate that in air its half-life is 20-50 minutes.[8] Gaseous ozone's efficacy increases with increased humidity. In the gaseous form, reaction rates of contaminants in the air are much slower, and adjustment of the pH is not possible. The process of using gaseous ozone typically involves pumping the ozone into a contained space, with an outlet where the excess ozone is destroyed.

Ozone is not expected to be used in gaseous form commercially (i.e., restaurants, bars, and mobile food services) due to the significant cost of equipment associated with controlling the application of the ozone and the protection of the workers applying the ozone. As such, only direct food applications in industrial settings are examined.

Use: Industrial Food Processing

Direct Food Application:

The highest concentration of ozone used as gaseous disinfectant in industrial food processing identified in literature was 754 mg/m^3 or 754 ppm, applied at a flow rate of 2 L/min for 119 minutes to treat rice grain.[5] The longest half-life identified was 13.80 minutes.

Equation 2 is overly conservative. An understanding of the chemistry of ozone indicates that even with this high concentration, the ozone would be expected to rapidly degrade. While the theoretical calculation from Equation 2 indicates that 16 hours would be necessary to decay the ozone, the empirical data from the study shows that the longest time observed for the complete decay of residual ozone was 61 minutes. With a decay time of 61 minutes, the Agency would have no expectation of residues in food by the time the food leaves the industrial food processing facility.

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[8] EPRI, Ozone as an Antimicrobial Agent for the Treatment, Storage and Processing of Foods in Gas and Aqueous Phases, Food Additive Petition filed with FDA, June 2000 (EPRI, 3412 Hillview Ave., Palo Alto, CA 94304).

Form: Aqueous Ozone

The half-life of ozone is highly dependent upon environmental conditions. Aqueous ozone is particularly sensitive to pH in addition to temperature. The Agency's National Secondary Drinking Water Regulations[9] list a guideline recommendation for drinking water pH of 6.5-8.5, which in pure water would result in a half-life for aqueous ozone of at most 3 hours.[10] However, under real-world conditions, ozone will readily react with most organic matter it contacts, reducing the half-life significantly. The Agency notes this in the Drinking Water Treatability Database website "Due to its short half-life, typically less than 30 minutes, a residual is not maintained in downstream processes; therefore, it can only be used as a primary disinfectant."[11] As such, for the sake of calculations, 30 minutes is assumed to be the half-life of aqueous ozone.

Aqueous ozone is expected to be used in both industrial and commercial use sites. Industrial and commercial use sites are typically assessed together. As noted in the Assumptions and Half-Life Calculations section above, indirect dietary exposures for industrial food processing are expected to remain negligible. However, commercial exposures are expected to have a significantly shorter time between application and consumption compared to industrial exposures. Therefore, both industrial food and commercial food processing use sites are considered separately for aqueous ozone.

Use: Industrial Food Processing

Direct Food Application: The solubility limit of ozone at room temperature, which is the highest concentration that room temperature aqueous ozone can be, is 8 mg/L, or 8 ppm.[2] The FAP reports using aqueous ozone at the solubility limit for direct food application disinfection in industrial food processing.[12]

Aqueous ozone applied directly to food is expected to come in contact with a large quantity of Natural Organic Matter (NOM); therefore, the half-life values determined for reaction of ozone with NOM-laden water is used. These values indicate that 50% of the ozone would be consumed within the first 10 seconds, and the subsequent half-life would be 5 minutes.[13] For ease of calculation, the initial residue is halved and a half-life of 5 minutes is used for calculations. Using Equation 2, and assuming a 5-minute half-life, it takes just under 5 hours for the ozone to degrade to a single molecule of ozone. Equation 2 assumes none of the ozone is removed through interaction with material surfaces, alkalinity, pH, etc., and is therefore an overly

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[9] EPA, Drinking Water Regulations and Contaminants, https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants Accessed May 8, 2020.

[10] Gardoni, D.; Vailati, A.; Canziani, R. (2012): Decay of Ozone in Water: A Review, *Ozone: Sci. Eng.: J. Int. Ozone Ass.*, **2012**, *34*, 233-242.

[11] EPA, Drinking Water Treatability Database, https://iaspub.epa.gov/tdb/pages/treatment/treatmentOverview.do?treatmentProcessId=-1467636837 Accessed May 8, 2020.

[12] EPRI, Membrane filtration and ozonation of poultry chiller overflow water: Study of membrane treatment to reduce water use and ozonation for sanitation at a poultry processing plant in California, *EPRI Technical Report TR-114435, Dec. 1999* (EPRI, 3412 Hillview Ave., Palo Alto, CA 94304).

[13] Westerhoff, P.; Song, R.; Amy, G.; Minear, R. Applications of Ozone Decomposition Models, *Ozone: Sci. Eng.: J. Int. Ozone Ass.*, **1997** ,*19*, 55-73.

conservative estimate of the amount of time it would take to degrade. Degradation of aqueous ozone under actual use conditions is expected to be much faster, as was the case for gaseous ozone above. Where Equation 2 predicted a 16 hour decay period for gaseous ozone, empirical data showed a decay time of 61 minutes, which suggests that the actual decay period for aqueous ozone is not 5 hours but less than one hour. This is expected from an understanding of the chemistry of ozone because the decay rate is expected to be faster than modeled due to the interactions with so many materials in the settings where ozone is used (this is not taken into account in the equations used). As such, the Agency would consider that food contact surfaces or food treated with aqueous ozone in industrial establishments would not reasonably be expected to result in ozone residues in food by the time the food leaves the industrial food processing facility.

Use: Commercial Food Processing

Indirect Food Application:

For commercial establishments such as restaurants, bars, and mobile food service vehicles, aqueous ozone formed using generally available commercial technology is typically applied at a concentration of no more than 4 ppm. An example of this use site is use of an ozonating faucet to clean dishes.

The Antimicrobials Division has a standard Tier 1 screening-level model used for dish detergents which estimates the pesticidal residue value remaining on dishes following a cleaning. This model assumes no degradation of ozone but rather that the ozone is diluted, and that 100% of the remaining ozone remains as a residue on the dishes.

The Tier 1 model for exposure (mg/day) for dish detergents is calculated with the following formula:

$$\boldsymbol{Residue\ (mg)} = \frac{\boldsymbol{F1}}{\boldsymbol{100}} \times \boldsymbol{C1} \times \boldsymbol{Ta} \times \boldsymbol{SA} \qquad \text{(Eq. 3)}$$

F1 = Percentage weight fraction of substance in product (%)
C1 = Concentration of product in dish wash solution (mg/cm^3)
Ta = Amount of water left on dishes after rinsing (ml/cm^2)
SA = Surface area of dishes in daily contact with food (cm^2)

Default Assumptions:

- C1 of 1 mg/cm^3
- Ta of 0.000055 mL/cm^2 (HERA (Human and Environmental Risk Assessment) project guidance, http://www.heraproject.com/)
- SA of 4,000 cm^2
- Density of solution of 1 g/mL

When 4 ppm aqueous ozone is applied, an estimated ozone residue of 8.80 x 10^{-7} mg residue is estimated. Dishwater is expected to contain large quantities of NOM, and so the half-life values determined for reaction of ozone with NOM-laden water is used. For ease of calculation, the residue value is halved to reflect that half of the ozone would decompose within ten seconds, and a half-life of 5 minutes is used for calculations. As such, the estimated residue value of ozone on the treated surface is 4.40 x 10^{-7} mg. Using these inputs results in a conservative time of 3.5 hours for complete removal. However, this calculation does not account for the high temperature used in dishwashing, the degradation which occurs during the washing, or decay on contact with the surfaces (plates, flatware, etc.). These factors alone would be expected to result in the entire removal of all residues of ozone by the time the dishwashing was complete.

The Antimicrobials Division also has a Tier 1 screening model to assess residues transferred to food as a result of commercial use on hard surfaces. The Tier 1 commercial screening model represents transfers to food over the course of a day from 4,000 cm^2 of treated surface, which is assumed to conservatively represent the amount of surface that food will come in contact with in a commercial food proceeding establishment.

The Tier 1 model for exposure (mg/day) for commercial hard surface uses is calculated with the following formula:

$$\boldsymbol{Residue\ (mg) = SA \times RS} \times \left(\frac{\boldsymbol{AR}}{\mathbf{1{,}000{,}000}}\right) \times \left(\frac{\boldsymbol{F}}{\mathbf{100}}\right) \qquad \text{(Eq. 4)}$$

SA = Area of Treated Surface (cm^2)
RS = Residual Solution (mg product/cm^2)
AR = Active Ingredient Concentration from product label, expressed as ppm
F = Fraction Transferred (%)
* 1,000,000 is a conversion factor

Default Assumptions:

- RS of 1 mg/cm^2 is a high-end assumption[14]
- SA of 4000 cm^2 is a high-end assumption based on commercial countertop food preparation surface area
- F is assumed to be 100%

In the absence of a product label, the same 4 ppm aqueous ozone concentration used in commercial settings is also used here. With these inputs, the Tier 1 screening-level model estimates 0.0160 mg is available on the hard surface to be transferred to food. As with dish detergents, the aqueous ozone is expected to come into contact with NOM, and so the residue value is halved to 0.0080 mg and a half-life of 5 minutes is used.

The Tier 1 screening model assumes that the calculated residue represents the maximum theoretical quantity of residue, found on a surface representing the maximum theoretical commercial surface (4000 cm^2), in contact with all food consumed over the course of an entire day. It does not represent the residue for any one meal for any one person, and it does not represent degradation of the pesticide, only dilution.

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[14] USFDA. Sanitizing Solutions: Chemistry Guidelines for Food Additive Petitions. Center for Food Safety and Applied Nutrition, V 1.1, January 1993.

When these values are inserted into Equation 2, the residue will require 4.7 hours to be entirely removed. However, as with the gaseous ozone, Equation 2 is overly conservative, and due to the aforementioned interactions, the residues would be expected to be completely removed in a fraction of the 4.7 hours calculated. Here, 0.0080 mg of ozone is spread over 4000 cm^2 and comes in contact with microbes and the surface itself, there is no expectation of residue on the treated surface.

Adjunct methods

While adjunct ozone is used in aqueous form, it is deliberately combined with another registered a.i. (typically hydrogen peroxide) as a reagent chemical to produce other oxidizers *in situ*, most notably to form hydroxyl radical. This process is seen as different enough from use of ozone as a direct disinfectant to justify examining its use separate from aqueous ozone.

Hydroxyl radical is an even more powerful oxidizer than ozone, and consequently has a half-life typically measured in microseconds. This process commonly occurs either through combining ozone with hydrogen peroxide in set ratios to form the hydroxyl radicals, or through combining UV radiation with ozone and water. While shorter wavelength UV light produces ozone, longer wavelength UV light destroys it. When the destroyed ozone reacts with the water, hydroxyl radicals are formed. Both of these reactions are fast and entirely consume the ozone in use. The adjunct form is more rapid in part because the first step in the mechanism of decomposition of aqueous ozone is reaction with hydroxyl ion, produced as a degradation product of hydrogen peroxide as shown in Equation 5.[6]

$$O_3 + OH^- \rightarrow O_2 + HO_2^- \qquad \text{(Eq. 5)}$$

In the same study used to test industrial aqueous ozone[8], aqueous ozone was combined with hydrogen peroxide in a 4:1 ratio. Disinfection results were similar, but only required an ozone concentration of 2 mg/L rather than 8 mg/L.

As the concentration of ozone in this adjunct method was 1/4th of the concentration of ozone in the aqueous ozone calculations, it is accepted that ozone combined with hydrogen peroxide in this manner would also result in no expectation of residues.

EPA/FDA Uses

Ozone is not registered as an active ingredient by the EPA, and the 1976 Devices FRN (40 C.F.R. § 162.15) specifically identifies ozone generators as devices and not subject to registration as pesticides. Ozone has been widely used in public drinking water treatment systems since the implementation of the 1973 Safe Drinking Water Act, administered by EPA's Office of Water. Ozone must be generated onsite and used immediately. Due to its short half-life, typically less than 30 minutes, a residual is not maintained in downstream processes; therefore, it can only be used as a primary disinfectant in drinking water. The product of ozone concentration (C) and contact time (T) determines CT, which is an important parameter in determining the ability of ozone to disinfect and inactivate microbes.

The FDA also has granted multiple clearances for residues of ozone in/on foods. Ozone is recognized under 21CFR 184.1563 *Direct Food Additives Generally Recognized as Safe (GRAS)* for use in bottled water.

Ozone is also listed under 21CFR 173.368 *Secondary Direct Food Additives Permitted In Food for Human Consumption,* which states ozone may be safely used in the treatment, storage, and processing of foods, including meat and poultry (unless such use is precluded by standards of identity in 9 CFR part 319), in accordance with the following prescribed conditions:

> (a) The additive is an unstable, colorless gas with a pungent, characteristic odor, which occurs freely in nature. It is produced commercially by passing electrical discharges or ionizing radiation through air or oxygen.
>
> (b) The additive is used as an antimicrobial agent as defined in 170.3(o)(2) of this chapter.
>
> (c) The additive meets the specifications for ozone in the Food Chemicals Codex, 7th ed. (2010), pp. 754-755, which is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. You may obtain copies from the United States Pharmacopeial Convention, 12601 Twinbrook Pkwy., Rockville, MD 20852 (Internet address http://www.usp.org). Copies may be examined at the Food and Drug Administration's Main Library, 10903 New Hampshire Ave., Bldg. 2, Third Floor, Silver Spring, MD 20993, 301-796-2039, or at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030 or go to: http://www.archives.gov/federal-register/cfr/ibr-locations.html.
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> (d) The additive is used in contact with food, including meat and poultry (unless such use is precluded by standards of identity in 9 CFR part 319 or 9 CFR part 381, subpart P), in the gaseous or aqueous phase in accordance with current industry standards of good manufacturing practice.
>
> (e) When used on raw agricultural commodities, the use is consistent with section 201(q)(1)(B)(i) of the Federal Food, Drug, and Cosmetic Act (the act) and not applied for use under section 201(q)(1)(B)(i)(I), (q)(1)(B)(i)(II), or (q)(1)(B)(i)(III) of the act.

Conclusion and FFDCA Clearance Determination

This memo has examined industrial and commercial uses of ozone in aqueous, gaseous, and adjunct forms. Given the highly reactive nature of ozone, its short half-life in both air and on surfaces, the lack of ozone residues remaining in/on surfaces, and the fact that ozone decomposes to oxygen, typical usage in industrial and commercial food processing facilities is reasonably expected not to result in residues of ozone in food. Therefore, no clearances under FFDCA Section 408 (tolerances or exemptions from the requirement of tolerances) are needed for the use of ozone directly applied to food or indirectly applied to food contact surfaces.