



Gas-phase Ozone: Assessment of Biocidal Properties for the Indoor Environment— A Critical Review

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Abstract

This report provides a detailed review of the scientific literature regarding the biocidal activity of gas-phase ozone. Many claims about its efficacy in reducing microbial contamination have been made by the manufacturers of ozone generators, which have unfortunately found their way into the popular press. However, this review of the peer-reviewed, published, scientific literature has found no appreciable antimicrobial effect of gas-phase ozone on either airborne or surface microorganisms. Its potential role in the control of biological pollution in the indoor environment is not substantiated by scientific investigations.

Introduction

This report addresses the biocidal activity of gas-phase ozone (O_3) in the indoor environment. While long used and recognized effective for the deodorization of smoke-damaged furnishings, recent commercial promotion of gas-phase ozone has purported, through aggressive marketing to consumers, to be effective at inactivating microorganisms, especially molds, in the air and on a variety of surfaces and materials in indoor environments at low concentrations (e.g., 0.04 ppm). A careful review of the published literature, both historically and more recently, however, provide substantial data to the contrary.

Manufacturers of gas-phase ozone generators, particularly those packaged as air cleaning devices,

lack the competent and reliable scientific evidence necessary to support far-reaching claims of killing airborne or surface molds with continuously generated low concentration ozone. Similarly, the use of high concentration ozone (>0.08 ppm) to effectively remediate (kill/inactivate) mold contamination on surfaces, such as within wall cavities, ceiling spaces, and HVAC ducts, remains unsupported by scientific evidence. Typically, such devices have never undergone valid, independent, and controlled laboratory efficacy testing, nor applied effectiveness testing in actual indoor environments, wherein the data generated were based on technically standardized, reproducible, and quality-controlled protocols deemed acceptable to the scientific community.

While high concentration (0.5-9.9 ppm) gas-phase ozone has been shown to inactivate some spore-forming and otherwise resistant microorganisms to some degree at high relative humidity (RH) on inert surfaces in the laboratory, as discussed in this review, there is no evidence of any degree of effectiveness on a variety of building materials in actual indoor environments. The recommended remediation of microbial contamination in indoor environments involves the physical removal of contaminated materials followed by thorough cleaning of the surrounding environment (Macher, 1999; USEPA, 2000). In particular, no studies confirm the antimicrobial efficacy of low- or high-concentration gas-phase ozone against microbial contamination on building, finishing, or furnishing materials in the indoor environment.

Scientific Background

Evaluation of Biocide Efficacy

A biocide may be defined as a chemical agent that inactivates (i.e., kills or prevents reproduction) of one or more groups of microorganisms, which includes bacteria, fungi, viruses, mycobacteria, and parasites. Efficacy may be defined as the demonstrated capability of an entity to produce a desired effect. Biocide efficacy is the capability of a liquid or gas-phase chemical to inactivate specific challenges of microorganisms under defined conditions. Efficacy is normally assessed in a controlled laboratory environment.

A variety of chemical, biological, and environmental factors, in combination, determine the efficacy of a particular biocide. These include, among others, chemical concentration, pH, temperature, relative humidity (RH), contact time, presence or absence of organic matter, surface or substrate composition, and microorganism type, quantitative challenge, and tendency toward monodispersion (singly separated cells, spores, viruses, or other units) or aggregation (clumping). Thus, efficacy of a biocide can only be defined under the specific set of circumstances under which it was tested. Efficacy may or may not relate to how the product actually performs as used in the real-world environment.

Efficacy of Gas-phase Ozone

The scientific literature addresses both aqueous ozone and gas-phase ozone. Both can be markedly different in terms of antimicrobial efficacy and potential application.

Aqueous ozone. Ozone dissolved in water is recognized as an effective biocide in low concentration against vegetative microorganisms such as bacteria, fungi, viruses, and parasites under ideal laboratory conditions (Hall and Sobsey, 1993; Wickramanayake, 1991). However, inactivation of these organisms in wastewaters where microbial concentrations are very high and there is considerable organic matter challenge requires larger doses of ozone and longer contact times.

Gas-phase ozone. Gas-phase ozone has been evaluated for its capability to inactivate microorganisms in the air and on surfaces. The survival or inac-

tivation of microbial aerosols is dependent upon a number of varied intrinsic and interrelated environmental factors (Cole & Cook, 1998).

Of great importance in this regard is the fact that airborne microorganisms or those on surfaces, whether bacterial cells, fungal spores, or virus particles, tend to occur most often in aggregates and typically in association with organic dust particles. These conditions provide a measure of protection from biocidal activity. Microbial clumping may preclude some cells or spores from having physical contact with the biocide. Likewise, organic matter may physically preclude biocide exposure and/or exert a neutralizing effect, depending upon its nature. This was dramatically demonstrated in a study where gas-phase ozone was introduced into flasks of actively growing *Achromobacter* bacteria (Ingram & Haines, 1949). Results showed that 1,000-2,000 ppm ozone had no effect on growth rate, 2,450 ppm began to affect the growth cycle, and 3,890 ppm was required to arrest all growth, with complete kill only after 30 hours exposure. Also exemplifying this concept, Elford and van Den Ende (1942) found that when airborne bacteria are covered with a protective coating of organic matter, as in aerosols naturally emitted during a sneeze or cough, gas-phase ozone at low concentration (0.2-0.3 ppm) had little effect. They also found that bacteria that settled onto surfaces are generally more resistant to ozone than when initially expelled from the respiratory tract.

The fact that microorganisms dried on a surface are intrinsically more resistant to germicidal inactivation forms the basis for microbial challenges dried onto steel or porcelain, as used in the standard Association of Official Analytical Chemists (AOAC) test methods required by the USEPA to generate efficacy data for the registration of chemical germicides. Also, it is well recognized that different types of microorganisms constitute a scale of innate resistance to inactivation, based upon their structural physiology and other unknown factors (Cole & Robison, 1996).

Biocidal Activity of Gas-phase Ozone

Well-controlled laboratory chamber studies sponsored by the USEPA have confirmed the lim-

ited efficacy of gas-phase ozone at high concentration and prolonged contact time against a variety of fungal and bacterial pollutants dried on surfaces (Foarde et al., 1997). Researchers first tested high concentrations (10^5 - 10^6) of mold spores, bacterial spores, and yeast cells, dried on glass surfaces. Samples were exposed continuously for 23 hours to ozone concentrations ranging from 0.5-9.9 ppm at 90% relative humidity (RH), and to 2.5-9.9 ppm at 30% RH. Yeast cell inactivation at 90% RH reached a 3 log (10-fold) reduction at 5.5 ppm, but inactivation was minimal at 30% RH. *Penicillium chrysogenum* and *P. glabrum* mold spores showed gradual inactivation dependent upon ozone concentration. At 90% RH a 3-log reduction was detectable at 6.2 ppm, while 30% RH required 9.8 ppm. The greatest challenge was shown to be the highly resistant spores of the *Streptomyces* bacterium, where negligible inactivation was observed at both high and low level RH, even at high concentration gas-phase ozone (7.3-9.9 ppm). Such results were similar to a previous study that investigated the inactivation of *Bacillus* spores on filter paper and glass fiber filters, and found that results of inactivation with 0.5-3.0 ppm ozone varied according to RH, with little inactivation occurring at RH of 50% or below (Ishizaki et al., 1986). Similarly, evaluation of inactivation of vegetative bacteria and yeast cells on stainless steel exposed to 2 ppm ozone for 4 hours showed varied kill, as well as diminished effectiveness in the presence of organic matter (Moore et al., 2000).

Of greatest significance is the second phase of the USEPA study, where researchers repeated the testing using mold spores of the two *Penicillium* species, RH of 90%, and the concentration of gas-phase ozone set to a level of 9 ppm. This time, however, the 10^5 - 10^6 concentrations of spores were not inoculated onto glass, but onto actual building materials to include two types of fiberglass ductliner, fiberboard duct, two types of ceiling tile, and galvanized steel. Even though exposure time remained at 23 hours, no appreciable inactivation of the spores on any of the materials was detectable compared to initial test concentrations. In fact, testing with the ceiling tiles would not permit gas-phase ozone concentrations in the chamber to rise above 5 ppm, demonstrating the neutralizing effect that materials can

have on gas-phase ozone at the microorganism/material interface.

Multiple Factors Limit Effectiveness

Effectiveness of a biocide is the measure of its efficacy in the real-world environment. Since gas-phase ozone has been shown to be ineffective in the laboratory under controlled conditions against a variety of microorganisms on actual building materials, effectiveness in an actual indoor environment under uncontrolled conditions would not be expected. Other factors against ozone's effectiveness include the inability of the gas to be delivered to all building spaces, difficulty in maintaining concentration and relative humidity over time, airflow and internal building pressures, and its reactivity with certain materials (such as rubber and electrical wire insulation with unsaturated carbon-carbon bonds).

A Critical Review of Often-cited Literature

Ozone-generating device manufacturers, while typically lacking effectiveness data for their units over and above natural microorganism death rates due to desiccation, lack of essential nutrients, or ultraviolet exposures, often cite various scientific published papers as evidence of the efficacy and/or effectiveness of gas-phase ozone. A closer look at such references, however, results in significantly different conclusions. For example, the papers "Ozone Inactivation of Cell-Associated Viruses" (Emerson et al., 1982), and "The Biological Effects of Ozone on Representative Members of Five Groups of Animal Viruses" (Bolton et al., 1982) have been pointed to for efficacy data for gas-phase ozone. The Emerson paper reports studies relevant to water and wastewater disinfection, as suspensions of cell-associated or unassociated viruses were ozonated at varying times and conditions. In fact, study data show a lack of ozone efficacy, as it is stated in the report that "in conclusion, cell-associated enteric viruses were protected from inactivation by exposure to ozone." A major issue is that the study was not an applied field evaluation of gas-phase ozone against naturally resistant or dried organisms in the indoor environment, but a laboratory evaluation of aqueous ozone efficacy. Similarly, the Bolton paper presents data relevant to the inactivation of virus suspensions using gas-phase ozone, re-

sulting again in an aqueous ozone situation not relevant to addressing active microbial growth or its dried residuals in the indoor environment.

Other cited papers present similar interpretation problems, such as "Ozone Decontamination of Bio-clean Rooms" (Masaoka et al., 1982). Here again, the challenges were wet organism suspensions on filter paper, in water, or soaked in a sponge, that actually represent aqueous ozone conditions rather than dried or actively growing microorganisms. In a survey paper, "Application of Ozone in Control of Mold, Bacteria, and Odors" (Nagy, 1957), ozone generated by an ultraviolet lamp for odor control is reviewed, and a determination made that heavy growths of mold and bacteria are not destroyed on surfaces. Also, studies in which a UV lamp is used to generate ozone make it difficult to distinguish germicidal effects from the ozone versus the UV itself, which is known to have antimicrobial properties.

Required Approach to Evaluation of Effectiveness

It must be remembered that while many studies in the scientific literature address the efficacy or effectiveness of ozone, the data generated in each represent the many, varied, and specific conditions under which the testing was done at that time. While such data are useful for general knowledge of, and in some cases specific applications for, ozone, one must be very cautious about taking selected data and extrapolating the results to any device that generates ozone. The only sure way to demonstrate that an ozone-generating device is effective as claimed is for test it independently, first for efficacy under controlled laboratory conditions and second for effectiveness under actual in-use or field conditions in the indoor environment, controlling all variables except those that are being investigated. Both laboratory and field studies must be designed and carried out according to concepts acceptable to the scientific community. This includes designing the protocols and conducting the work in such a fashion that the study can easily be reproduced by other investigators.

Microbial Residuals

Of great concern with the use of any biocide is the residual that remains if microorganisms are

killed or otherwise inactivated. Residuals of killed bacteria and fungi are still regarded as potential indoor pollutants. When gram-negative bacteria are killed and their cell walls are disrupted, those fragments are known as endotoxin—highly reactive molecules which may become airborne and be inhaled. Endotoxins are very stable and can be reliably destroyed only by extreme heat. They are recognized as causing pulmonary inflammation and irritation, and potentiating the allergic response (Rylander & Jacobs, 1994). Endotoxin exposures have been implicated in diseases such as byssinosis and humidifier fever. Likewise, mold spores that have been killed can still retain intact antigens capable of inciting an allergic response in a sensitized individual. Gas-phase ozone has never been demonstrated to destroy or otherwise neutralize such mold antigens.

In addition to molds, some air-cleaning device manufacturers may tout the efficacy of gas-phase ozone to destroy, neutralize, or inactivate a variety of other allergens, such as those from dust mites or cats, although no published study data have been identified. At the present time, the most rational approach indicates that periodic cleaning (extraction) and routine maintenance (high-efficiency vacuuming) of carpet and upholstery, as well as similar attention to mattresses, along with indoor moisture control, will reduce dusts generated from those sources and consequently the allergens associated with them (Cole et al., 2000; Franke et al., 1997).

Health Effects Claims

No positive health effects resulting from the application of gas-phase ozone to an indoor environment have ever been reported. On the contrary, ozone is a major respiratory irritant, wherein as little as 0.08 ppm exposure can result in cough, chest discomfort, lung inflammation, and increased airway reactivity (Cotran et al., 1999). Current national health standards for ozone exposure include the EPA's National Ambient Air Quality Standard of a maximum 8-hour average concentration of 0.08 ppm, while the National Institute for Occupational Safety and Health (NIOSH) recommends that an upper limit of 0.10 ppm not be exceeded at any time, with a threshold of 5.0 ppm considered imme-

diately dangerous to life or health (NIOSH, 2003). In this regard, there are no recommendations for specific respirator cartridges deemed adequate to protect workers from exposure and potential health effects from high concentration gas-phase ozone.

Manufacturers utilizing product marketing claims that state or imply a positive human health effect resulting from its use, such as “allows occupants to breathe easier,” “reduces numbers and severity of respiratory infections,” or similar claims, should be required to prove such claims through a randomized, controlled, clinical trial in which biological and other markers of health are monitored for a lengthy period in both intervention and control groups. Such studies minimally require 18-24 months or more to complete, are overseen by physicians, and require a detailed study design approved by an Institutional Review Board for the protection of human subjects. Biological and health markers typically include daily peak flow measurements, number of asthmatic attacks, number of physician and hospital visits, use of prescription medications, etc. While typically costing in excess of \$1 million, such studies are the only way to provide meaningful evidence or not of whether regular and long-term use of the ozone-generating product or device results in positive health effects.

Conclusion

In summary, a review of the peer-reviewed, published, scientific literature has shown no appreciable antimicrobial effect of gas-phase ozone on either airborne or surface microorganisms, relative to significant control of biological pollution in the indoor environment. In general, considering the extent of variability of types and concentrations of biological pollutants, their spectrum of intrinsic inactivation resistances, their range of recognized reservoirs, and the extreme variability in environmental conditions relative to temperature, RH, air flow, and organic matter interference among others, there remains no recognized antimicrobial effectiveness nor recommended protocol for the use of gas-phase ozone for the indoor environment.

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