EPA/600/R-08-137
December 2007

Ozone Antimicrobial Efficacy

By

Karin Foarde and Cary Eaton, Ph.D.
RTI International
3040 Cornwallis Road
P.O. Box 12194
Research Triangle Park, NC 27709-2194

GSA Contract No. GS-10F-0283K Order EP05C000057
RTI Project No. 09432.001

Project Officer

Marc Menetrez, Ph.D.
U.S. Environmental Protection Agency
National Risk Management Research Laboratory
Research Triangle Park, NC 27711

Notice

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development (ORD) managed the research described in this report. The research was performed by RTI. The report has been subjected to the Agency‘s peer and administrative review and has been approved for publication as an EPA document. Any opinions expressed in this report are those of the author and do not, necessarily, reflect the official positions and policies of the EPA. Any mention of products or trade names does not constitute recommendation for use by the EPA.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation’s land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA’s research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency’s center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory’s research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL’s research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory’s strategic long-term research plan. It is published and made available by EPA’s Office of Research and Development to assist the user community and to link researchers with their clients.

Sally C. Gutierrez, Director
National Risk Management Research Laboratory

Abstract

Ozone is a potent germicide that has been used extensively for water purification. The germicidal activity of ozone in water has been reported by many authors (see for example U.S. EPA, 1999); however, there is limited information on the biocidal activity of ozonated air as a treatment for contaminated surfaces. Understanding of the biocidal capability of ozone against the microorganisms primarily responsible for indoor air quality biocontaminant problems is still relatively limited.

In a previous research project, we evaluated the biocidal efficacy of three to 10 ppm ozone on selected microorganisms in laboratory chamber studies under controlled conditions (Foarde et al., 1997). The organism challenge consisted of vegetative organisms or spores dried on surfaces. The study was conducted in two phases. First, an extensive series of tests employing glass slides as the test surface were performed under ideal conditions of ozone exposure in which intensive efforts were made to minimize (or eliminate) ozone losses in the chambers. Second, a short series of tests was performed using building materials as the test surfaces. We found that ozone concentrations of 6 to 10 ppm were required to obtain 3-log reductions in colony-forming units (CFUs). The results from the second phase of the study, where spores of *Penicillium spp*. were deposited on actual building material surfaces, showed no reduction in CFUs after a 23-hr exposure to 9 ppm of ozone. For the denser materials (ceiling tiles), test levels of ozone were not attained, probably due to the reaction with the substrate.

The objective of this project was to expand on work from the earlier study by testing the effect of ozone at much higher levels (up to 1000 ppm for 24 hours) on a variety of microorganisms. The goal of these experiments was to ascertain the biocidal efficacy of ozone against four organisms − two bacteria (one spore and one vegetative organism) and two fungi (one spore and one vegetative organism). A series of experiments was performed using either glass slides or gypsum wallboard as the test surface. This series of experiments confirmed the results of the earlier experiments that the organisms on glass slides were more readily killed than organisms on building materials for higher levels of ozone. It would be reasonable to assume that the difference in ozone efficacy between the two test surfaces was due at least in part to the ability of the gypsum wallboard to inactivate the ozone and thus to protect the spores deposited on its surface. Also as in the earlier experiments, increasing RH increases the biocidal capability of ozone.

Because adverse health effects differ by organism and susceptibility of the exposure population, no standard acceptable level of contamination exists, nor does any required level of efficacy for decontaminating building materials in the field, therefore, a key issue in evaluating the efficacy of any biocide, including ozone, is to determine the acceptable number of CFUs remaining after treatment. For example, in these experiments the inoculum was usually at least 1×10^6 CFU. A 1 log reduction (90% inactivation efficiency) would mean that 1×10^5 CFU remained after exposure. If a 4 log reduction (99.99% inactivation efficiency) was attained, there would be 100 CFUs remaining after exposure. The acceptability of either of these inactivation efficiencies would depend on the specific situation.

Although the specific results vary depending upon the test organism and the test surface, the overall results of this study indicate that, even at relatively high concentrations of ozone, it is difficult to achieve significant inactivation of organisms on material surfaces. The high ozone concentrations used in this study would probably be difficult to maintain near or at the surface of some commonly contaminated building materials, and even if these concentrations could be maintained in the field, it would be challenging to achieve a significant reduction of surface biocontamination using ozone.

Table of Contents

Section **Page**

List of Figures

Figure **Page**

List of Tables

Table **Page**

1. Introduction

Ozone is a potent germicide that has been used extensively for water purification (Weavers and Wickramanayake, 2001). In Europe, 90 percent of the municipal water systems are treated with ozone, and in France, ozone has been used to treat drinking water since 1903. The germicidal activity of ozone in water has been reported by many authors (see for example U.S. EPA, 1999); however, there is limited information on the biocidal activity of ozonated air as a treatment for contaminated surfaces. Ozone has long been used as an effective deodorant in the remediation of smoke-damaged buildings. As an important reactive species in the atmosphere, the chemistry of ozone and volatile organic compounds has also been widely studied (Atkinson and Carter, 1984.)

Understanding of the biocidal capability of ozone against the microorganisms primarily responsible for indoor air quality biocontaminant problems is still relatively limited. In a previous research project, we evaluated the biocidal efficacy of three to 10 ppm ozone on selected microorganisms in laboratory chamber studies under controlled conditions (Foarde et al., 1997). The organism challenge consisted of vegetative organisms or spores dried on surfaces. The study was conducted in two phases. First, an extensive series of tests employing glass slides as the test surface were performed under ideal conditions of ozone exposure in which intensive efforts were made to minimize (or eliminate) ozone losses in the chambers. Second, a short series of tests was performed using building materials as the test surfaces. We found that ozone concentrations of 6 to 10 ppm were required to obtain 3-log reductions in colony-forming units (CFUs). The results from the second phase of the study, where spores of *Penicillium spp*. were deposited on actual building material surfaces, showed no reduction in CFUs after a 23-hr exposure to 9 ppm of ozone. For the denser materials (ceiling tiles), test levels of ozone were not attained, probably due to the reaction with the substrate.

The objective of the project discussed here was to expand the early work by testing at much higher ozone levels. The goal of these experiments was to ascertain the effect of ozone against two bacteria (a vegetative bacterium and a bacterial spore) and two fungi (a vegetative fungus and a fungal spore) dried on the surfaces of glass microscope slides and pieces of gypsum wallboard. The target ozone levels were 100, 500, and 1000 ppm. Three exposure times, 1.5 hr, 6 hr, and 24 hr, were used.

In addition, tests were performed at two levels of relative humidity (RH). Many factors, including temperature, pH, RH, and organic load, affect the susceptibility of microorganisms to ozone (Foegeding and Busta, 1991). Increasing RH increases the biocidal capability of ozone (Clark and Takács, 1980). In addition, materials in buildings are exposed to a range of RHs. Two ranges of RHs were selected for use in this study: low RH (20–45%) and high RH (80–95%). These two levels bracket the use conditions for ozone in most buildings and provide information at two extreme air moisture situations.

2. Methods and Materials

2.1 Ozone Exposure Apparatus

2.1.1 Exposure Chamber

A commercially available desiccator cabinet constructed of polished stainless steel and glass was used to carry out the microorganism exposure experiments. A schematic of the ozone apparatus and setup is shown in **Figure 1**. In earlier experiments (Foarde et al., 1997), the all-glass chamber interior was coated with Teflon: the coating was important to limit ozone reactivity with surfaces at the low ozone concentrations (1 to 10 ppm) being used. The experiments described here used much higher ozone concentrations (100 to 1000 ppm), and measurements to assess the stability of these higher concentrations within the chamber demonstrated that there was no need to line the chamber with Teflon for this study because the

higher levels of ozone, flowing constantly thorough the chamber at various fixed rates from 15 to 27 liters per minute, could be maintained throughout the chamber interior at all chosen exposure times. The chamber has interior dimensions of approximately 30 x 25 x 30 (cm, L x W x H) and a total volume of approximately 22 liters. For use as an ozone exposure chamber, the chamber interior was accessed through an airtight door lined with a neoprene gasket. Humidified air containing ozone flowed through a plastic bulkhead fitting mounted in the chamber's rear wall, 3 cm from the bottom. A sample for the ozone analyzer was withdrawn continuously at a flow rate of 1.0 liter per minute through Teflon tubing mounted in a bulkhead fitting in the center of the rear wall; the remainder of the flowing ozone-containing air exited to the laboratory hood through a fitting and tubing attached 3 cm from the top of the chamber's rear wall. Uniformity of ozone concentrations at various points within the chamber showed the air was homogeneously mixed. Inoculated microscope slides and glass Petri dishes containing inoculated wallboard samples were placed on two to four perforated stainless steel shelves within the chamber in such a way that ozone-bearing air could circulate freely around the samples.

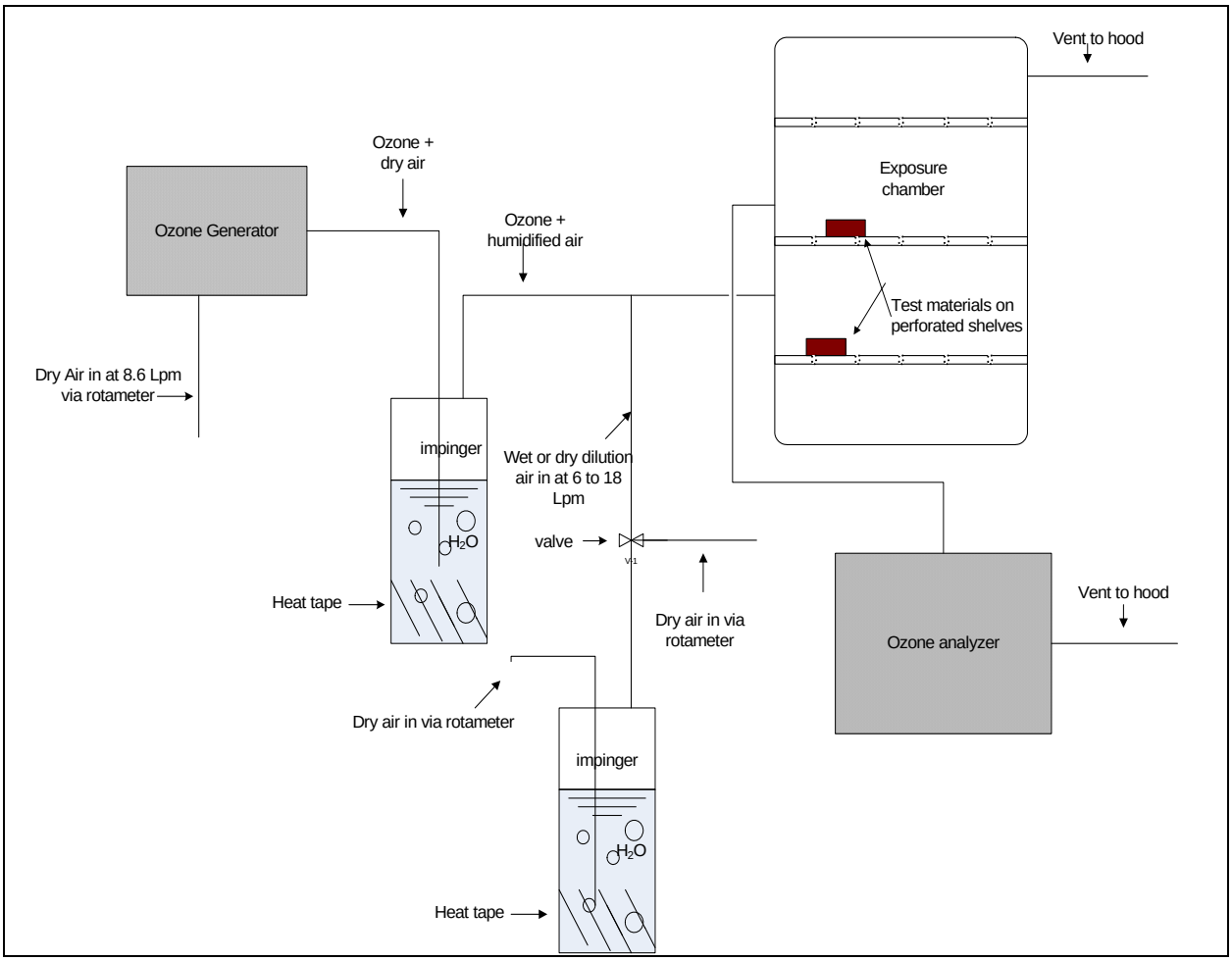

Figure 1. Schematic of the test apparatus.

During set up of the ozone generator and exposure chamber, a TECO Model 49 ambient air ozone analyzer was used to characterize the system. Ozone-laden air, nominally 500 ppm, flowing at several liters per minute through the exposure chamber, was diluted 1:1000 with clean air to allow detection by the TECO 49 analyzer. The ozone-containing air entered through a port at the bottom of the rear wall of the exposure chamber. Ozone/air vented from the exposure chamber from a port at the center of the chamber's back wall (and then diluted 1:1000) registered 447 ppb on the TECO 49. Another sample taken from a port near the top of the chamber also registered 447 ppb. Therefore, we concluded that the mixing

and flow conditions within the exposure chamber were such that the ozone concentration was spatially uniform. In all exposure experiments, ozone entered the chamber at the bottom of the back wall and exited at the top (i.e., through the vent). The ozone-laden air had to pass around and through the perforated stainless steel shelves on which the test samples were placed.

2.1.2 Air Supply and Humidifier

The test air for the experiments was ozonized and humidified using the apparatus shown in Figure 1. Dry, particle-free, compressed house "zero" air was used. The clean air stream was split into two streams using rotameters and needle valves. One air stream passed through the ozone generator and thence to an all-glass impinger containing deionized water. The concentration of the ozone and the RH were controlled by the introduction of dilution air that was also humidified by passage through a separate impinger. The two airstreams combined at a "tee" and entered the chamber. The exteriors of the glass impingers were wrapped in heating tape so that the temperature of the water remained constant and thus the amount of water vapor delivered to the chamber remained nearly constant. The tubing through which sampled air was pulled to the temperature and RH sensor was placed in the chamber exhaust port before and after ozone production. Room temperature, controlled by the building's heating, ventilation, and air conditioning (HVAC) system was monitored at a point adjacent to the chamber. The air flowed through the chamber as described above.

Temperature and humidity were measured by a factory-calibrated EdgeTech Model 2000 Series DewPrime dew point hygrometer. To protect the humidity sensor from high ozone concentrations, measurements of exposure chamber temperature (T) and RH were made on air withdrawn from the chamber prior to introduction of ozone and immediately after ozone exposure ceased; all conditions (air flow, humidification, temperature) were identical except for the production of ozone by the generator.

2.1.3 Ozone Source

A Model GTC-0.5 ozone generator (Ozonia North America, Griffin Division) was used to generate ozone by corona discharge. The generator's ozone output was controlled by varying the amperage; ozone concentration was further controlled by varying the dilution air flow.

The ozone concentration in the chamber was measured using a Teledyne Instruments/Advanced Pollution Instrumentation Ozone Monitor, Model 460M. The factory-calibrated instrument operated on a 0–1000 ppm range. The accuracy and precision of the ozone monitor were estimated to be +/- 10 ppm. The voltage signal output from the ozone monitor was recorded by a laboratory data acquisition system that averaged the signal over 10-minute periods and expressed the results as ppmV. The accuracy of the Teledyne ozone monitor's response was confirmed indirectly as follows:

- First, the response of a TECO Model 49 ambient ozone monitor, operating on the 0 to1 ppm (0 to 1000 ppb) range was shown to be accurate and linear by challenging it with ozone generated and verified by a TECO 49C ozone primary standard calibrator: we challenged the TECO 49 using the primary standard at seven concentrations ranging from 0 to 879 ppb, and the TECO 49 responses agreed well with the primary standard photometer designated values (within 1.56 percent or better at all seven concentrations). The response of the Model 49 (C_{49}, in ppb) was related to the response of the Model 49C (C_{49c}, in ppb) as follows: $C_{49} = 1.0044*C_{49c}+1.408$ ($r^2 = 1.0000$).
- Next, high concentrations of ozone in air were diluted so that they could be accurately sensed by the TECO Model 49 ambient monitor and simultaneously compared to the values reported by the Teledyne Model 460M high-concentration ozone monitor. To accomplish this, the exposure chamber was set to 50% RH, and nominal 800 ppm ozone was produced for an exposure time of 180 minutes. Then, the TECO 49 and Teledyne 460M monitors sampled from the same location within the chamber.

The TECO 49 responses (after accounting for 1:1000 dilution) averaged 725.1 ± 41.5 ppm [5.7 percent relative standard deviation (RSD)], based on 14 readings taken over the 180 minute period. The Teledyne 460M responses averaged 789.8 ± 3.5 ppm (0.44 percent RSD), based on 18 readings taken over the same time period. The agreement of the Teledyne and the TECO average concentrations was within ~ 8.8 percent, which met the data quality indicator goal of 10 percent accuracy of ozone concentration determination. The precision of the ozone measurements for various ozone exposure levels and time frames were most often within ± 10 ppm. The higher standard deviation for the TECO 49 responses was likely due to the dilution process and small variations in flow-regulating components. The excellent standard deviation shows the temporal stability of ozone in the exposure system as well as the advantages of an ozone monitor that does not require sample dilution. In conclusion, the factory calibration of the Teledyne Model 460M was sufficiently accurate. Ozone concentrations for all exposures shown in the final report were monitored by the Teledyne Model 460M.

Following verification of the accuracy of the Teledyne monitor's response, the chamber was characterized as follows:

1. Set up the chamber for use, including test gas entry, exit, and sampling ports, placement of support shelves within the chamber, and placement of test surfaces (glass microscope slides and glass Petri dishes holding squares of gypsum wallboard).
2. Establish a steady-state ozone concentration at the low end of the test range~~,~~ i.e., 100 ppm.
3. Measure ozone concentration in the center of the chamber to ensure there is no variability at a single location.
4. Repeat the measurement at various locations within the chamber to ensure the test gas was well-mixed as indicated by steady ozone readings.
5. Fine tune the air flow rates, the water-containing impinger conditions, and ozone generator output to achieve the desired ozone concentration and RH for each experiment.

2.2 Test Organisms

The four organisms selected for testing in this study were *Rhodotorula mucilaginosa*, *Penicillium brevicompactum*, *Bacillus atrophaeus*, and *Staphylococcus epidermidis*. *B. atrophaeus* and *S. epidermidis* are bacteria, and *R. mucilaginosa* and *P. brevicompactum* are fungi (a yeast and a mold, respectively). Yeasts are single-celled organisms that reproduce by budding and do not form spores. Molds, however, are composed of long branching filaments called hyphae (a mass of hyphae is referred to as a mycelium). Mycelia or hyphae are the vegetative phase of the organism. Molds reproduce by forming spores that are resistant to unfavorable environmental conditions and can remain dormant for long periods of time. Only the spores of *P. brevicompactum* were used in this study.

B. atrophaeus is a gram-positive, spore-forming bacterium. In this study, only the spore of *B. atrophaeus* was used. The vegetative bacterium was *S. epidermidis*. Because vegetative organisms are generally more susceptible to the effect of biocides than spore-forming organisms, and bacterial spores usually more resistant than fungal spores, *S. epidermidis* was expected to be the most susceptible and the *B. atrophaeus* spore, the least susceptible.

Three of the test organisms were purchased from the American Type Culture Collection (ATCC): *P. brevicompactum* (ATCC #9056), *B. atrophaeus* (ATCC #9372), and *S. epidermidis* (ATCC #12228). *R. mucilaginosa*, a field isolate from a contaminated building site being maintained in the RTI culture collection (RTI CC #3435), was identified using the Biolog MicroLog Microbial Identification System (Biolog, Inc., Hayward, CA.). The Biolog system is a commercially available product for broad-based rapid identification and characterization. This system uses redox chemistry based on the reduction of tetrazolium, which responds to the metabolism of specific substrates by the test organism.

2.3 Microorganism Challenge

The form of the challenge was a critical element in the design of the study. Typically, biocides are evaluated using the most difficult test conditions under which they may be expected to function. For this study, the test organisms were dried on the surface of gypsum wallboard pieces or on glass slides, because destruction of organisms dried on surfaces is a difficult challenge for a biocide and because building surfaces such as gypsum wallboard are frequent targets for ozone treatment during remediation. The number of challenge organisms was based on levels of contamination that have been reported on surfaces in buildings. Levels ranging from 10^3 to 10^7 CFU/cm^2 have been isolated from surfaces of contaminated buildings ranging from ceiling tile to wallboard (Morey, 1993).

2.4 Experimental Procedures

These experiments used two types of test surface: glass microscope slides and gypsum wallboard pieces. Each test included seven individual test surfaces (glass slides or pieces of gypsum wallboard). For each test, one of the test organisms was suspended in water and measured volumes were pipetted onto each of the seven test surfaces and allowed to dry. The seven test surfaces were dried, and four of the surfaces exposed in the test chamber as described above. The remaining three surfaces, kept on the bench adjacent to the test chamber, served as unexposed controls (which provided both a baseline and a measure of inoculum viability). Following exposure, the exposed test surfaces and the control test surfaces were processed, and the effects of the ozone evaluated by comparing the CFUs of the exposed and unexposed surfaces. The test conditions are given in **Table 1.**

Table 1. Summary of Test Conditions for Each Test Organism and Each Test Surface Type

Relative Humidity	Ozone Concentration	Exposure Times		
Low (20–45%)	1000 ppm	1.5 hr	6 hr	24 hr
	500 ppm	1.5 hr	6 hr	24 hr
	100 ppm	1.5 hr	6 hr	24 hr
	0 ppm	1.5 hr	6 hr	24 hr
High (80–95%)	1000 ppm	1.5 hr	6 hr	24 hr
	500 ppm	1.5 hr	6 hr	24 hr
	100 ppm	1.5 hr	6 hr	24 hr
	0 ppm	1.5 hr	6 hr	24 hr

The test matrix included the following elements:

- Two test surface types, glass microscope slides and gypsum wallboard
- Seven test surfaces for each run (3 controls and 4 exposed)
- $10^3 – 10^7$ CFUs/test surface
- Four different microorganisms(fungal spore, bacterial spore, vegetative bacteria, and yeast)

2.4.1 Quantitative Evaluation

Each of the seven test surfaces was placed in a separate container, suspended in sterile phosphate buffered saline (PBS) containing Tween 80, and shaken for at least five minutes. All necessary dilutions were made using the same buffer. Aliquots of the suspension were plated on the appropriate media and incubated for the optimal time and temperature for the test organism. CFUs were counted and calculated for each test surface piece.

3. Results

3.1 Comparison of Number of CFUs from Exposed and Unexposed Test Pieces

The effects of the ozone on the test organisms were evaluated by comparing the number of CFUs recovered from the exposed slides or wallboard to those recovered from the unexposed test surfaces. CFUs were counted, numbers per glass slide or wallboard piece computed, and the data transformed to their logarithmic (base 10) value. The averaged results of the three control or four exposed samples, including standard deviations, are shown in Tables 2 through 5 by organism. **Table 2** shows the results for glass slides at low RH. **Table 3** shows the results for gypsum wallboard at low RH. **Table 4** shows the results for glass slides at high RH, and **Table 5** shows the results for gypsum wallboard at high RH. All of the tables have the same structure: The first column shows the ozone concentration (ppm) in the air flowing through the test chamber; the second column shows the time that the glass slides or gypsum wallboard pieces were exposed in the chamber; the third column shows the calculated total ozone exposure (the ozone concentration [C, in ppm] was multiplied by the time [T, in minutes] to determine the exposure [C*T, in ppm-minutes]); and the last two columns show the mean and standard deviation of the number of CFU $\log_{10}$ test organisms on the control or exposed test pieces.

The minimum detection limit for the test organism was 150 CFU per sample based on the analysis method described in Section 2. Therefore, BDL (below detection limit) is used in the tables when fewer than 150 CFUs were isolated from the exposed samples. (Note that the tables present the $\log_{10}$ of CFU; the $\log_{10}$ of the 150 CFU detection limit is 2.18.)

Table 2. CFUs for Control and Exposed Samples on Glass Slides at Low Humidity

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU $\log_{10}$) Mean ± St. Dev.	Exposed Samples (CFU $\log_{10}$) Mean ± St. Dev.
P. brevicompactum				
119.4 ± 22.1	90	10,746	5.92 ± 0.04	5.95 ± 0.10
504.6 ± 14.5	90	45,414	6.61 ± 0.05	6.10 ± 0.31
516 ± 9	360	185,760	6.79 ± 0.21	2.46 ± 0.57
501.8 ± 13.6	1440	722,592	6.34 ± 0.08	BDL*
1007 ± 20.7	90	90,630	5.19 ± 0.46	4.88 ± 0.33
1010 ± 13.4	360	363,600	5.80 ± 0.17	BDL
R. mucilaginosa				
119.4 ± 22.1	90	10,746	4.48 ± 0.30	5.07 ± 0.36
504.6 ± 14.5	90	45,414	7.20 ± 0.08	7.10 ± 0.04
516 ± 9	360	185,760	7.46 ± 0.03	7.53 ± 0.04
501.8 ± 13.6	1440	722,592	7.08 ± 0.08	6.77 ± 0.10
1006 ± 20.7	90	90,540	7.70 ± 0.05	7.45 ± 0.09
1010 ± 13.4	360	363,600	7.70 ± 0.05	6.88 ± 0.21
B. atrophaeus				
119.4 ± 22.1	90	10,746	6.47 ± 0.25	6.71 ± 0.36
504.6 ± 14.5	90	45,414	6.36 ± 0.07	6.44 ± 0.43
516 ± 9	360	185,760	6.62 ± 0.11	6.58 ± 0.18
501.8 ± 13.6	1440	722,592	6.40 ± 0.06	6.06 ± 0.34
1007 ± 20.7	90	90,630	5.85 ± 0.29	5.92 ± 0.19
1010 ± 13.4	360	363,600	6.60 ± 0.12	7.02 ± 0.12

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU log_{10}) Mean ± St. Dev.	Exposed Samples (CFU log_{10}) Mean ± St. Dev.
1014 ± 17.8	1440	1,460,160	6.43 ± 0.17	6.28 ± 0.13
S. epidermidis				
119.4 ± 22.1	90	10,746	7.00 ± 0.28	6.60 ± 0.25
504.6 ± 14.5	90	45,414	7.15 ± 0.13	4.54 ± 0.53
516 ± 9	360	185,760	5.47 ± 0.13	2.66 ± 0.35
501.8 ± 13.6	1440	722,592	6.72 ± 0.43	BDL
1007 ± 20.7	90	90,630	5.72 ± 0.32	4.49 ± 0.57
1010 ± 13.4	360	363,600	5.72 ± 0.32	BDL

* BDL – Below Detection Limit of 2.18 CFU log_{10}

Table 3. CFUs for Control and Exposed Samples on Gypsum Wallboard at Low Humidity

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU log_{10}) Mean ± St. Dev.	Exposed Samples (CFU log_{10}) Mean ± St. Dev.
P. brevicompactum				
119.4 ± 22.1	90	10,746	7.13 ± 0.16	6.61 ± 0.36
504.6 ± 14.5	90	45,414	6.61 ± 0.05	6.87 ± 0.08
516 ± 9	360	185,760	7.37 ± 0.02	7.43 ± 0.09
501.8 ± 13.6	1440	722,592	7.32 ± 0.09	5.61 ± 0.66
1007 ± 20.7	90	90,630	5.80 ± 0.37	5.97 ± 0.10
1010 ± 13.4	360	363,600	6.56 ± 0.15	6.37 ± 0.22
1014 ± 17.8	1440	1,460,160	6.75 ± 0.04	5.46 ± 0.52
R. mucilaginosa				
119.4 ± 22.1	90	10,746	7.05 ± 0.18	7.27 ± 0.19
504.6 ± 14.5	90	45,414	6.62 ± 0.11	6.85 ± 0.25
516 ± 9	360	185,760	7.36 ± 0.09	7.44 ± 0.04
501.8 ± 13.6	1440	722,592	6.62 ± 0.22	6.98 ± 0.63
1006 ± 20.7	90	90,540	7.06 ± 0.20	6.76 ± 0.27
1010 ± 13.4	360	363,600	7.06 ± 0.20	6.28 ± 0.13
B. atrophaeus				
119.4 ± 22.1	90	10,746	6.41 ± 0.17	6.15 ± 0.28
504.6 ± 14.5	90	45,414	6.48 ± 0.21	6.49 ± 0.21
516 ± 9	360	185,760	6.54 ± 0.10	6.94 ± 0.54
501.8 ± 13.6	1440	722,592	6.72 ± 0.59	6.88 ± 0.41
1007 ± 20.7	90	90,630	6.09 ± 0.11	5.70 ± 0.11
1010 ± 13.4	360	363,600	6.63 ± 0.16	6.29 ± 0.14
1014 ± 17.8	1440	1,460,160	6.55 ± 0.36	6.44 ± 0.13
S. epidermidis				
119.4 ± 22.1	90	10,746	4.58 ± 0.46	4.35 ± 0.52
504.6 ± 14.5	90	45,414	6.34 ± 0.81	6.20 ± 0.51
516 ± 9	360	185,760	5.21 ± 0.50	5.20 ± 0.62
501.8 ± 13.6	1440	722,592	6.71 ± 0.47	5.28 ± 0.63

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU log_{10}) Mean ± St. Dev.	Exposed Samples (CFU log_{10}) Mean ± St. Dev.
1006 ± 20.7	90	90,540	6.44 ± 0.71	6.08 ± 0.62
1010 ± 13.4	360	363,600	6.44 ± 0.71	5.79 ± 0.50

Table 4. CFUs for Control and Exposed Samples on Glass Slides at High Humidity

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU log_{10}) Mean ± St. Dev.	Exposed Samples (CFU log_{10}) Mean ± St. Dev.
P. brevicompactum				
100.2 ± 6.15	90	9,018	5.81 ± 0.13	3.10 ± 1.41
509.6 ± 5.9	90	45,864	6.61 ± 0.27	2.18 ± 0
506 ± 5	360	182,160	6.19 ± 0.06	BDL
1003 ± 6.9	90	90,270	5.19 ± 0.46	BDL
R. mucilaginosa				
100.2 ± 6.15	90	9,018	4.51 ± 0.35	4.57 ± 0.43
509.6 ± 5.9	90	45,864	4.18 ± 0.00	2.88 ± 0.92
506 ± 5	360	182,160	7.39 ± 0.02	5.69 ± 0.06
B. atrophaeus				
100.2 ± 6.15	90	9,018	6.60 ± 0.24	6.69 ± 0.23
509.6 ± 5.9	90	45,864	6.42 ± 0.15	3.94 ± 0.40
506 ± 5	360	182,160	6.62 ± 0.03	BDL
1003 ± 6.9	90	90,270	5.85 ± 0.29	3.67 ± 0.17
966 ± 20.5	360	358,560	6.63 ± 0.19	2.30 ± 0.24
S. epidermidis				
509.6 ± 5.9	90	45,864	6.12 ± 0.07	BDL

BDL – Below Detection Limit of 2.18 CFU log_{10}

Table 5. CFUs for Control and Exposed Samples on Gypsum Wallboard at High Humidity

O_3 concentration (ppm)	Exposure time (min)	Total Exposure (ppm-min)	Control Samples (CFU log_{10}) Mean ± St. Dev.	Exposed Samples (CFU log_{10}) Mean ± St. Dev.
P. brevicompactum				
100.2 ± 6.15	90	9,018	6.74 ± 0.01	6.34 ± 0.80
509.6 ± 5.9	90	45,864	6.79 ± 0.56	4.66 ± 0.48
506 ± 5	360	182,160	7.17 ± 0.21	BDL
1003 ± 6.9	90	90,270	5.80 ± 0.37	3.62 ± 0.31
966 ± 20.5	360	358,560	6.58 ± 0.04	BDL
R. mucilaginosa				
100.2 ± 6.15	90	9,018	7.13 ± 0.16	6.76 ± 0.08
509.6 ± 5.9	90	45,864	7.06 ± 0.17	6.56 ± 0.18
506 ± 5	360	182,160	7.32 ± 0.09	BDL
B. atrophaeus				
100.2 ± 6.15	90	9,018	6.76 ± 0.08	6.40 ± 0.08
509.6 ± 5.9	90	45,864	6.54 ± 0.39	6.46 ± 0.53
506 ± 5	360	182,160	6.59 ± 0.16	3.11 ± 1.08
1003 ± 6.9	90	90,270	6.09 ± 0.13	5.70 ± 0.15
966 ± 20.5	360	358,560	6.52 ± 0.05	BDL
S. epidermidis				
509.6 ± 5.9	90	45,864	6.12 ± 0.07	BDL

BDL – Below Detection Limit of 2.18 CFU log_{10}

*3.2 Log Change in CFUs for the Total (C*T) Ozone Exposure*

To quantify the effectiveness of the ozone to inactivate the test organisms, the log change in CFUs was plotted against the total ozone exposure (C*T). The log CFUs of either the three replicate inoculated, unexposed controls or the four replicate inoculated exposed slides were averaged and the standard deviation calculated. The log change was calculated as follows:

$$Log\ change = Log\,CFU_C - Log\,CFU_E \text{ where}$$

Log CFU_E = mean log CFUs of exposed samples (n=4)
Log CFU_C = mean log CFUs of control samples (n=3).

Figures 2 through **5** show the results for each of the test organisms for both test materials at both RHs. The X-axis is the range of ozone exposure (C*T) in ppm ozone-min. The Y-axis is the log change. The error bars are the combined standard error of the mean of the exposed and control samples for each test. When the Log CFU for the exposed samples was BDL, the value 2.18 CFU log_{10} was used to calculate the Log change.

As anticipated, the test organisms were more protected on the gypsum wallboard and at low RH. On glass slides at low RH, no effect was observed for the *B. atrophaeus* and *R. mucilaginosa*, while the *S. epidermidis* and *P. brevicompactum* both decreased at least 4 logs at the maximum ozone exposure. However, at high RH on both glass slides and gypsum wallboard, all of the organisms but the *R. mucilaginosa* were inactivated 4 logs. On the gypsum wallboard at low RH, none of the organisms was inactivated as much as 2 logs.

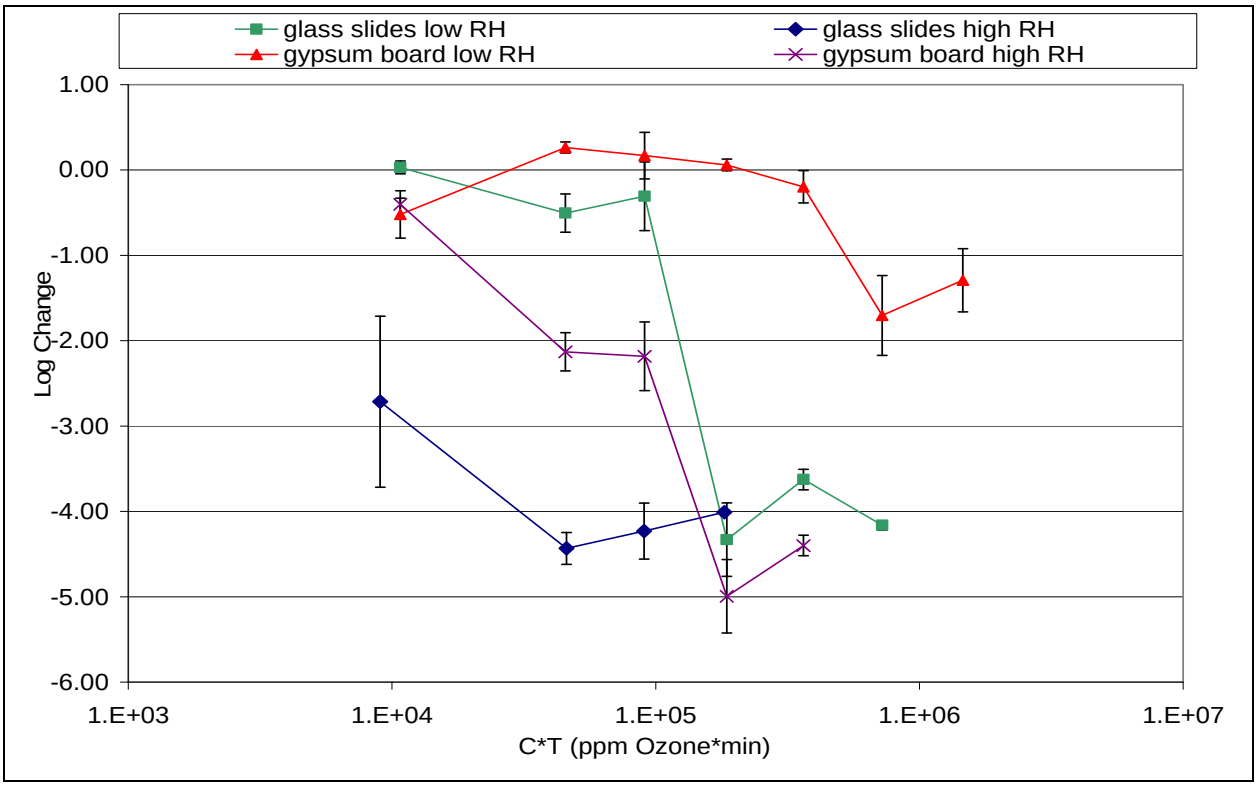

Figure 2. Log change in CFU of *P. brevicompactum* over a range of ozone exposure.

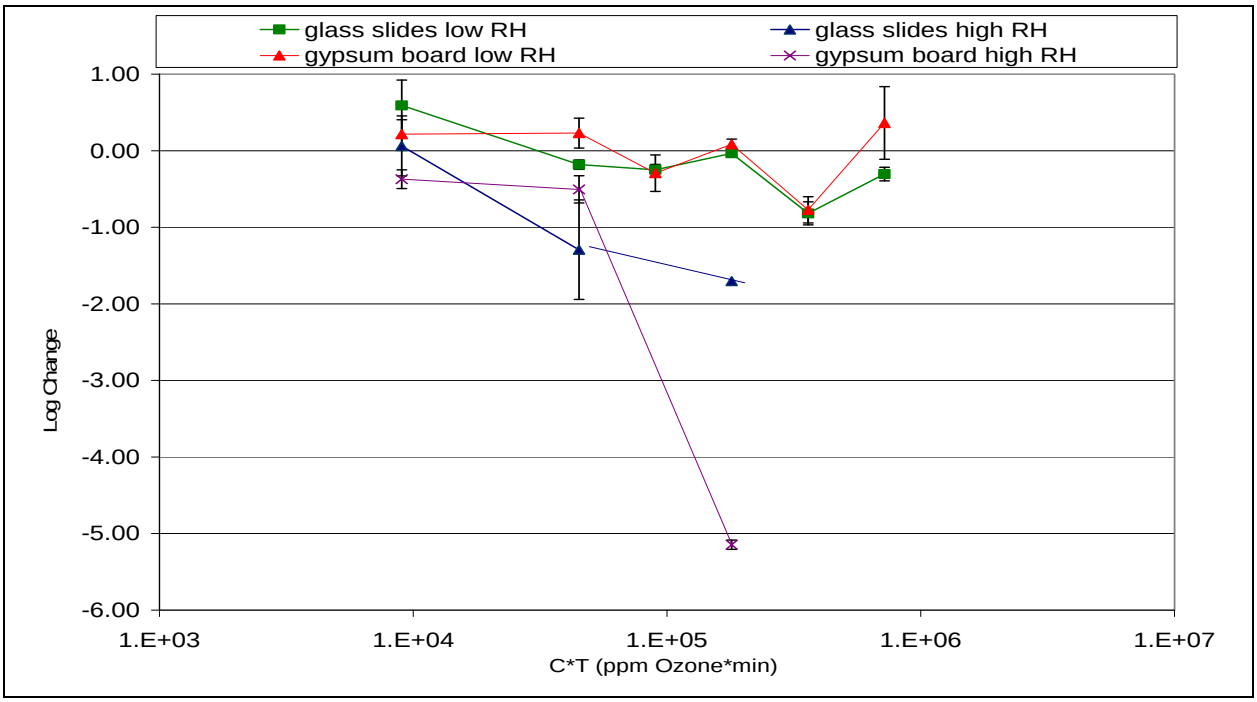

Figure 3. Log change in CFU of R mucilaginosa over a range of ozone exposure.

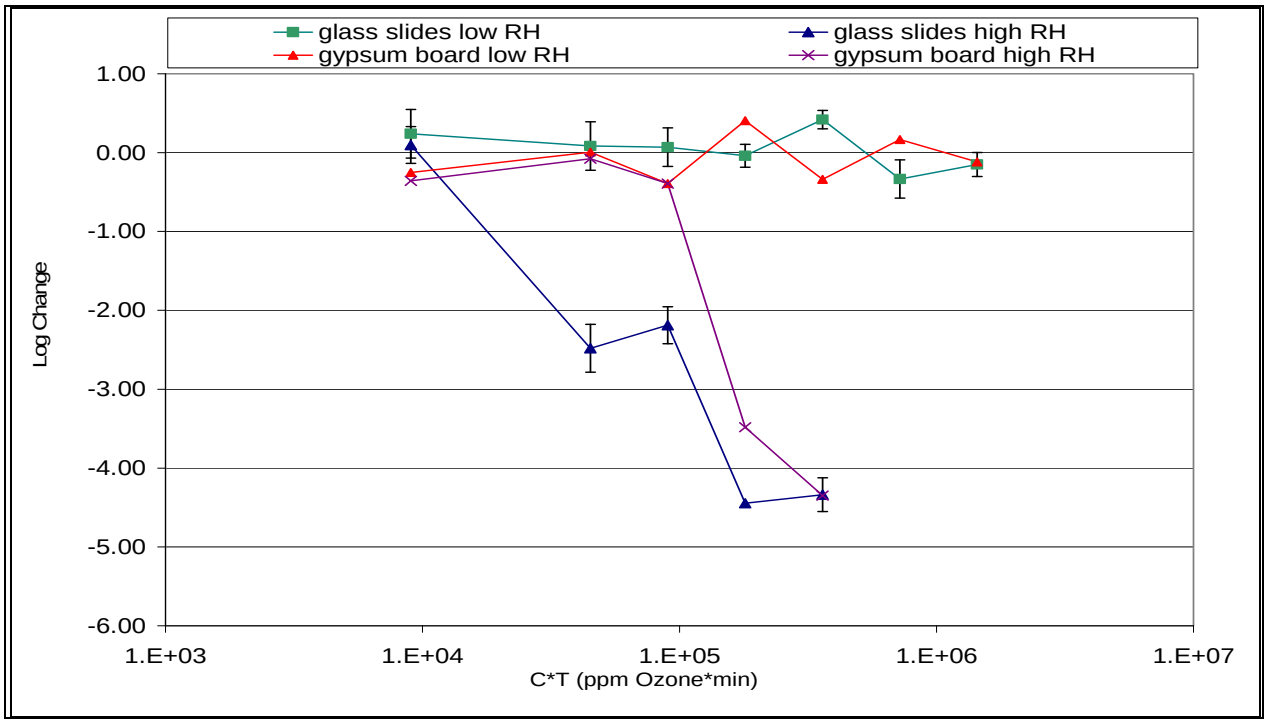

Figure 4. Log change in CFU of *B. atrophaeus* over a range of ozone exposure.

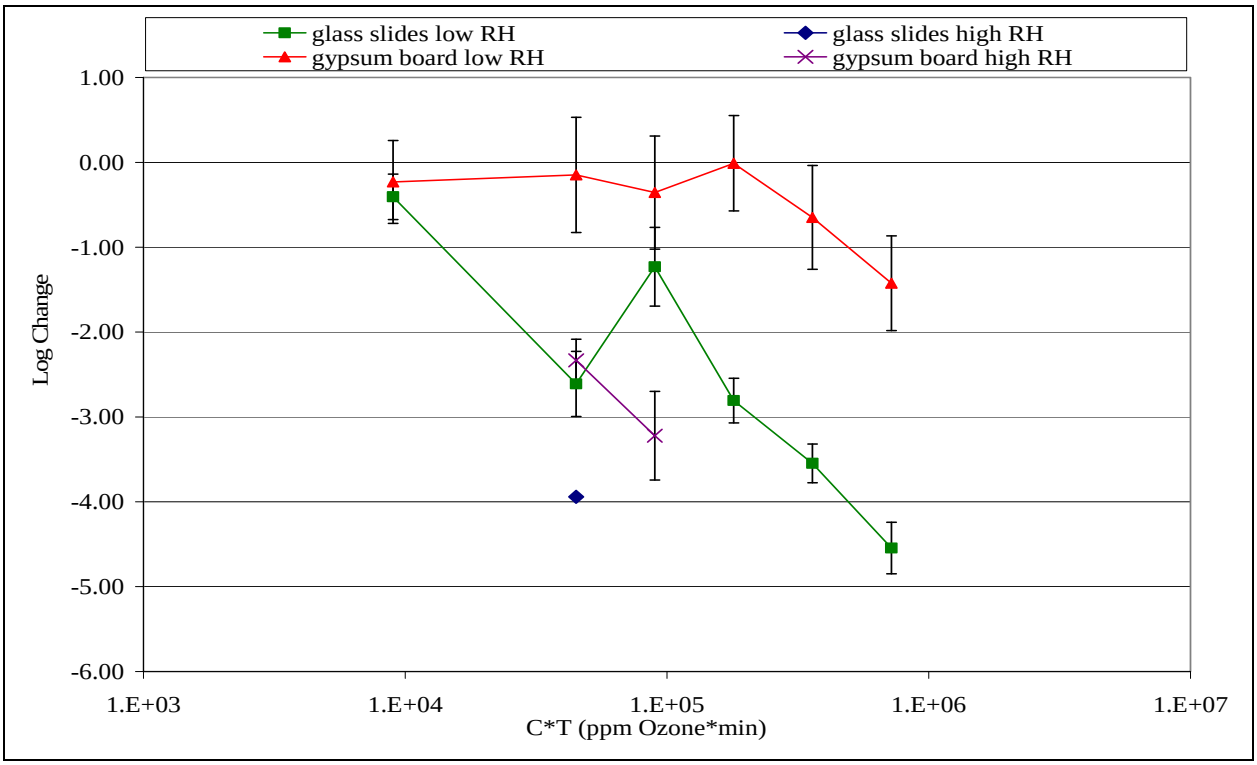

Figure 5. Log change in CFU of *S. epidermidis* over a range of ozone exposure.

4. Discussion and Conclusions

The objective of this project was to expand on work from an earlier study (Foarde et al., 1997) by testing the effect of ozone at much higher levels on a variety of microorganisms. The goal of these experiments was to ascertain the biocidal efficacy of ozone against four organisms − two bacteria (one spore and one vegetative organism) and two fungi (one spore and one vegetative organism). In the earlier study, we evaluated the effects of ozone at levels up to 9 ppm. In this study we evaluated levels ranging from 100 to 1000 ppm (Menetrez et al., 2008).

A series of experiments was performed using either glass slides or gypsum wallboard as the test surface. The use of the impenetrable, flat-surfaced glass slides was to minimize the loss of the ozone at the surface where the test organisms were deposited, thereby maximizing the probability of detecting an effect. However, in order to evaluate ozone under more realistic use conditions, the test organisms were also inoculated onto the back surfaces of an actual building material, gypsum wallboard.

In earlier experiments at lower levels of ozone, we found that the organisms on glass slides were more readily killed than organisms on building materials. This series of experiments confirmed those results for higher levels of ozone. It would be reasonable to assume that the difference in ozone efficacy between the two test surfaces was due at least in part to the ability of the gypsum wallboard to inactivate the ozone and thus to protect the spores deposited on its surface.

As stated earlier, increasing RH increases the biocidal capability of ozone. This was found to be true in the earlier, low-level study and was confirmed in this study at higher ozone levels.

Because adverse health effects differ by organism and susceptibility of the exposure population, no standard acceptable level of contamination exists, nor does any required level of efficacy for decontaminating building materials in the field, therefore, a key issue in evaluating the efficacy of any biocide, including ozone, is to determine the acceptable number of CFUs remaining after treatment. For example, in these experiments the inoculum was usually at least 1×10^6 CFU/sample. A 1 log reduction (90% inactivation efficiency) would mean that 1×10^5 CFU remained after exposure. If a 4 log reduction (99.99% inactivation efficiency) was attained, there would be 100 CFUs remaining after exposure. The acceptability of either of these inactivation efficiencies would depend on the specific situation.

Although the specific results vary depending upon the test organism and the test surface, the overall results of this study indicate that, even at relatively high concentrations of ozone, it is difficult to achieve significant inactivation of organisms on material surfaces. The high ozone concentrations used in this study would probably be difficult to maintain near or at the surface of some commonly contaminated building materials, and even if these concentrations could be maintained in the field, it would be challenging to achieve a significant reduction of surface biocontamination using ozone.

5. References

Atkinson, R. and W. P. L. Carter. 1984. Kinetics and Mechanisms of the Gas-Phase Reactions of Ozone with Organic Compounds under Atmospheric Conditions. *Chemical Reviews*. 84(5): 440–469.

Clark, D.S. and J. Takács. 1980. Gases as Preservative. In: *Microbial Ecology of Foods. Factors Affecting Life and Death of Microorganisms.* International Commission on Microbiological Specification of Foods. New York Academic Press. 1:170–192.

Foarde, K., D. VanOsdell, and R. Steiber. 1997. Investigation of Gas-Phase Ozone as a Potential Biocide. *Appl. Occup. Environ. Hyg*. 12(8):535–542.

Foegeding, P.M. and F.F. Busta. 1991. Chemical Food Preservatives. Chapter 47 in: *Disinfection, Sterilization and Preservation*. Fourth Edition. S.S. Block, ed. Philadelphia: Lea and Febiger.

Menetrez, M.Y., Foarde, K.K., Schwartz, T.D., Dean, T.R., Betancourt, D.A., 2009. An Evaluation of the Antimicrobial Effects of Gas-Phase Ozone, Journal of Ozone: Science and Engineering.

Morey, P. R. 1993. Use of Hazard Communication Standard and General Duty Clause During Remediation of Fungal Contamination. In: *Indoor Air '93 Proceedings* 4:391-395.

U.S. EPA (Environmental Protection Agency). 1999. *Wastewater Technology Fact Sheet: Ozone Disinfection*. Report Number EPA 832-F-99-063. Prepared by the Office of Water, Washington, DC. September. Available at http://www.epa.gov/owmitnet/mtb/ozon.pdf.

Weavers, L.K. and G.B. Wickramanayake, 2001. Disinfection and Sterilization by Ozone. Chapter 10 in: *Disinfection, Sterilization, and Preservation*. Fifth Edition. S. Block, ed. Philadelphia: Lippincott Williams and Wilkins.