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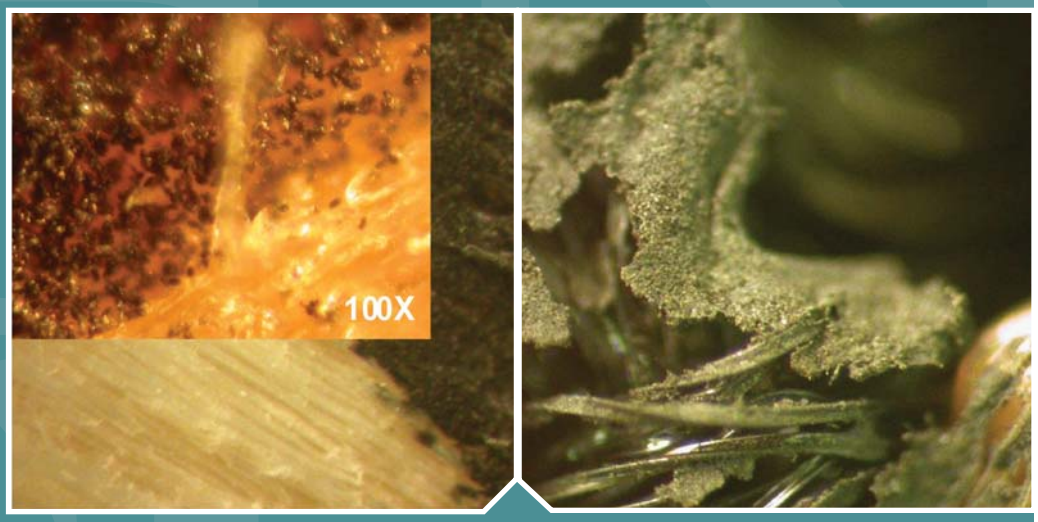
Laboratory Evaluation of the Efficacy of Chlorine Dioxide Fumigation for Remediation of Building Materials Contaminated with Molds, Mycotoxins or Allergens



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Laboratory Evaluation of the Efficacy of Chlorine Dioxide Fumigation for Remediation of Building Materials Contaminated with Molds, Mycotoxins or Allergens

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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ACRONYMS AND ABBREVIATIONS

Ag	antigen
Asp f1	*Aspergillus fumigatus* antigen
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
B.a.	*Bacillus anthracis*
CFU	colony forming unit(s)
ClO_2	chlorine dioxide
CRADA	Cooperative Research and Development Agreement
CM	Critical measurement
CT	concentration of chlorine dioxide X time of exposure
DAS	digital acquisition system
DQI	Data quality indicator
DQO	Data quality objective
ELISA	enzyme-linked immunosorbent assay
EMS	environmental monitoring system
ERH	Equilibrium Relative Humidity
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FTTA	Federal Technology Transfer Act
hr	hour(s)
HVAC	heating, ventilation and air conditioning
MSM	Modified Standard Method
ng	nanogram(s)
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
ORIA	Office of Radiation and Indoor Air
PBS	phosphate buffered saline
PCR	polymerase chain reaction
P&DC	Processing and Distribution Center
ppmv	part(s) per million by volume
QAPP	Quality Assurance Project Plan
R&D	research and development
T	temperature
rAlt a 1	*Alternaria* antigen
RH	relative humidity
RTI	RTI International
SJRMC	Saint Johns Regional Medical Center
USEPA	United States Environmental Protection Agency

EXECUTIVE SUMMARY

The purpose of this project was to determine the efficacy of chlorine dioxide (ClO_2) fumigation to inactivate viable mold, mycotoxins, and allergens on building materials. *Alternaria alternata*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Chaetomium globosum*, and *Stachybotrys chartarum* were individually inoculated onto the surface of latex-painted and wallpapered gypsum wallboard, unpainted pine wood, ceiling tile, carpet, and glass. In addition, neat preparations of one allergen (rAlt a1extract from *Alt. alternata*) and one mycotoxin (aflatoxin) were spiked onto surfaces in pure form. Spiked building materials were fumigated with ClO_2 gas at low and high concentrations for several time periods. The analytical techniques included counting colony forming units (CFU) for molds and use of enzyme-linked inmunosorbent assays (ELISA) for quantification of the remaining antigen and mycotoxin. A ≥ 4 $\log_{10}$ reduction in CFU was achieved after exposure at 9000 parts per million by volume (ppmv)-hour (hr) at 75 °F and a relative humidty of 75% for *S.chartarum*, *C.globosum*, *A. versicolor*, *A. fumigatus* and *A. alternata* on latex-painted wallboard, unpainted pine wood, carpet and glass. A ≥ 4 $\log_{10}$ reduction in CFU was achieved for *S. chartarum* on all materials and all ClO_2 concentration x exposure time (CTs) from 3000 to 9000 ppmv-hr.

Allergens and mycotoxins were partially inactivated under several of the conditions tested.

This research has demonstrated the potential of ClO_2 gas to achieve a large percentage reduction in viable spore counts on all materials as well as a significant percentage reduction of the concentration of allergens and mycotoxins. Observations of fumigation of actual buildings with heavy mold contamination are consistent with these results.

1. INTRODUCTION

Mold contamination is frequently cited as the causative agent in a variety of health problems (IOM, 2004; Andersen et al., 2011; Dearborn et al., 1999). In instances of extensive or heavy contamination, mold contamination can also lead to expensive and time-consuming efforts to remediate the problem using conventional approaches. This report summarizes the results of research under a Cooperative Research and Development Agreement (CRADA) to quantify the efficacy of fumigation using chlorine dioxide (ClO_2) for inactivation of a variety of common mold species on typical building materials.

The objective of this study was to determine the biocidal efficacy of ClO_2 fumigation against a variety of fungi (molds) and their nonviable components, e.g., allergens and mycotoxins. The organisms chosen for this study are representative of mold species commonly associated with damp indoor environments (Vesper et al., 2007). In particular, *C. globosum* was tested at multiple exposure concentrations and exposure times because this organism is highly resistant to adverse environmental conditions. This mold produces a multilayered spore that confers protection against UV light and desiccation (Millner et al., 1977). Likewise, the mold has been demonstrated to offer protection against oxidation by ClO_2 (Wilson et al., 2005).

Mold contamination of structures

Mold spores are ubiquitous in natural and built environments and, under normal circumstances, are relatively innocuous (Frankel et al., 2012). However, under wet/high moisture conditions favorable to the formation of the vegetative form, mold has been associated with human health issues and damage to the impacted structures (IOM, 2004; Vesper et al., 2006; Gravesen et al., 1999). The effects on human health may result from the proliferation of spores, contact with the vegetative organism and/or production of allergens and mycotoxins. Mold spore contamination may occur as the result of water damage associated with normal day-to-day occurrences or with widespread damage from natural disasters.

Conventional approach to mold remediation

The industry standard for mold remediation is described in the United States Environmental Protection Agency (USEPA) report entitled “Mold Remediation in Schools and Commercial Buildings” (USEPA, 2001). Because that approach is beyond the scope of this report, only the broad outline will be provided as follows:

- Assess the extent and cause of the problem.
- Repair structural damage causing water leaks.
- Remediate other sources of moisture.
- Remove waterlogged contents (e.g., carpet, bedding), if appropriate.
- Determine if full or partial containment and ventilation are necessary to prevent spread of mold during remediation activities.
- Remediation may entail selective removal of water-damaged finish materials (e.g., wallboard, ceiling tile). However, in the case of pervasive water damage, the structure

may be gutted to the structural elements (e.g., studs and joists) and the electrical and heating, ventilation and air conditioning (HVAC) systems removed.
- Dry out the affected area(s) of the structure.
- Sample to determine the effectiveness of the remediation.

In cases of extensive water damage, the approach can be time consuming and expensive, especially in a material and labor constrained market (such as after a natural disaster).

Alternative approaches

In the aftermath of Hurricane Katrina, several vendors offered alternative approaches to mold remediation that were claimed to be more effective or less disrupting than the conventional approach. Sabre Technical Services, LLC, offered the ClO_2 fumigation technique, which was used successfully in response to the 2001 "anthrax" incidents (Martin, 2005) and will be discussed in greater detail in the following section. Sabre received state level registration for mold remediation under Section S4(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in Louisiana, Texas and Mississippi. The approach was applied to structures with residual issues that had been remediated conventionally and to structures with extensive to pervasive mold growth that had not previously been remediated. In summary, the approach was as follows:

- Apply the technique after structural and other sources of water were remediated.
- Remove carpet and other water-saturated materials.
- Tent the structure and fumigate with ClO_2; remediating structure and contents *in situ.*
- Ensure fumigant penetration into all parts of the structure (e.g., attic, wall cavities).
- Vacuum to remove residue once the fumigation is complete.
- While some contents showed material effects (e.g., discoloration of materials or oxidation of mild steel) and required disposal, many items were suitable for reclamation/reuse.

Federal Technology Transfer Act

The Federal Technology Transfer Act (FTTA) grants the USEPA the authority to make its experimental facilities and expertise available to non-Federal organizations to perform research and development (R&D) of mutual interest. The FTTA allows the non-federal cooperator to provide both funding and in-kind resources to the Federal organization. The Federal organization can provide a variety of in-kind resources for performance of the R&D but may not provide resources to the cooperator. The CRADA is the mechanism for formalizing the scope of the R&D to be conducted collaboratively between the Federal organization and the cooperator. The R&D described in this report was completed under a CRADA between Sabre Technical Services, LLC (henceforth Sabre) and the USEPA's Office of Research and Development (ORD), with additional resources from the USEPA's Office of Pesticide Programs (OPP). The goals of the CRADA were:

- Evaluate a range of ClO_2 fumigation conditions to determine if a 4 log_{10} reduction of viable spores could be achieved.
- Evaluate the effects of ClO_2 fumigation on mycotoxins and allergens.

As a part of the CRADA, USEPA performed the fumigation using its in-house fumigation research facility in Research Triangle Park, NC.

Background

A number of techniques for disinfection or sterilization of microbes have been developed for a variety of applications. When the 2001 “anthrax” incidents occurred, many approaches were used to remediate the numerous buildings that were affected. One such approach was fumigation with ClO_2, which has been suggested as a method for remediation of buildings contaminated with mold. One method of ClO_2 generation that uses a wet method to generate ClO_2 from the reaction of bleach, hydrochloric acid and sodium chlorite to generate ClO_2 was developed by John Y. Mason *et al.* (1993). This approach was used at the Hart Senate Office Building and the Brentwood Processing and Distribution Center (P&DC), both in Washington, D.C., and the Hamilton P&DC in NJ (Canter, 2005; Martin, 2003). The conditions that were used required a minimum CT of 9000 ppmv-hr (750 ppmv ClO_2 for 12 hours) at a minimum 75% relative humidity (RH) and 75 °F. This same approach has been used at two fumigations for mold in contaminated structures: an abandoned Victorian era house in Utica, NY, and an abandoned department store building in Hudson Falls, NY. The conditions that were used were 9000 ppmv-hr CT (3000 ppm for 3 hours). This approach was also used to remediate the Saint Johns Regional Medical Center (SJRMC) for mold at a lower CT of 2000 ppmv-hr to minimize material impacts (Martin et al., 2008). The conditions developed in these previous tests were a starting point for the work contained in this report.

2. MATERIALS AND METHODS

Overall technical approach. The laboratory evaluation was designed to simulate some of the conditions that could be found in mold-contaminated structures. The materials were selected to represent the most common materials used in building and home construction. The mold species selected are usually identified in contaminated structures. The overall approach consists of four components, as follows:

- Fumigation experiments to evaluate the efficacy of ClO_2 gas as a function of concentration, time, overall CT and RH.

- Culturability Assay. The inactivation of culturable fungi was quantified by comparing the number of colony forming units (CFU) pre- and post-treatment on the material coupons.

- Mycotoxin Analysis. The inactivation of aflatoxin was measured by an enzyme linked immunosorbent assay (ELISA).

- Allergen Analysis. The inactivation of purified *Alternaria* antigen (rAlt a1) was quantified by direct ELISA. In addition, the inactivation of rAlt a1 from the *Alternaria alternata* CFU inoculated coupons and Asp f1 from *Aspergillus fumigatus* CFU inoculated coupons was quantified.

Coupon Preparation. The building material coupons were prepared using standard procedures as summarized below. All coupon preparations and analyses were performed at RTI International, Research Triangle Park (RTP), NC.

- *Coupon Materials*. The coupon materials were selected to represent commonly used building materials known to be subject to mold growth. These materials were bare structural pine wood (Home Depot, Raleigh, NC), latex-painted gypsum wallboard (Home Depot, Raleigh, NC), wallpapered latex-painted gypsum wallboard (Home Depot, Raleigh, NC), carpet (Kraus, Brentwood Carpets, Raleigh, NC), ceiling tiles (Home Depot, Raleigh, NC), and glass (Prism Research, Raleigh, NC). Wood and ceiling tile are porous materials that support mold growth not only on the surface but also penetrating into the coupon. Painted and wallpapered gypsum wallboard has a relatively nonporous surface yet is quite porous internally. If water compromises the surface (i.e., makes the surface porous), then painted and wallpapered gypsum is a perfect substrate for mold growth. Mold growth on carpet is dependent on the age and the composition of the carpet and is subject to spore contamination. Smooth glass was selected as a control material for biological studies because mold growth was not expected. Most building materials exhibit properties that might interfere with the ClO_2 inactivation of biological contaminants. However, glass, being a smooth hard surface, eliminates any chemical interference related to material composition.

The coupons were 1 inch by 1 inch squares cut from the interior portion of the source sample to ensure that all coupons had uniform characteristics. The coupons were individually autoclaved prior to inoculation with the target organism. Contaminants (mold spores, mycotoxins or allergens) were inoculated onto the surface of the six building materials. Test coupons were exposed to ClO_2 at different concentrations. Positive control coupons were prepared like the test coupons but were not exposed to the ClO_2. The negative control coupons were not inoculated but were exposed to ClO_2 with the test coupons. Field blanks (not inoculated and not exposed) were also included.

Two types of coupons were prepared – vegetative coupons and nonvegetative coupons. The mold spores were inoculated on the surface of the vegetative coupons, and the coupons were placed in a static chamber for at least six weeks to allow active growth to occur. The inactivation of growing fungi was determined for the latex-painted gypsum wallboard, the wallpapered gypsum wallboard, the ceiling tile and the wood. Inoculation was performed in a Class II Biosafety Cabinet (BSCII). The cabinet was decontaminated prior to each use. The fungi challenge suspensions were prepared by inoculating the test organism onto solid agar media (MMBD Standard Operating Procedure (SOP) #001), incubating the culture at room temperature until mature, wiping organisms from the surface of the pure culture, and eluting the organisms into sterile deionized water (MMBD SOP #002) to a known concentration to serve as a stock solution (MMBD SOP #012). The organism preparation was viewed microscopically to verify the purity of spores (absence of hyphae). The suspension was diluted in sterile deionized water when needed. The coupons were inoculated (usually with five 10 to 100-µL spots in an X configuration) by pipet onto the surface of the coupon and allowed to dry in the biosafety cabinet (MMBD SOP #58).

Clear plastic desiccators served as the static environmental chambers. The desiccators have gasket-sealed doors, which eliminate air exchange and maintain the humidity within the chamber through the use of sterile water. Temperature was externally controlled and maintained at room temperature. Prior to use, the chambers were decontaminated (MMBD SOP #005) and then characterized as per MMBD SOP #017. The chambers were set to 100% Equilibrium Relative Humidity (ERH) for all tests. The ERH in each chamber was monitored with a hygrometer (Vaisala, Woburn, MA) .

Prior to fumigation, the challenge level of CFU on vegetative coupons was determined by assaying coupons to determine the level of growth. CFU eluted were compared to the baseline inoculum at Day 0. Growth was defined by an increase of approximately 1 $\log_{10}$ and confirmed visually by microscopy. The goal was to load each of the individual coupons with 10^6 to 10^7 CFU/coupon. However, the nature of *C. globosum* growth (forms perithecia) and *Alt. alternata* growth (produces few very large spores) made achieving 10^6 to 10^7 CFU/coupon difficult. Modifications to the level of the inoculum were necessary. By lowering the minimum detection limit, the log reduction was able to be measured with an appropriate dynamic range without achieving the high level of inoculum.

Fungal spores were inoculated on the surface of the nonvegetative mold coupons (without an incubation period) and the coupons were fumigated. Nonvegetative coupons included all of the

carpet and glass coupons, the wood *Stachybotrys* and *Alternaria* coupons, and all of the allergen and mycotoxin inocula. Carpet and glass did not support growth of the test organisms because the surfaces were not soiled prior to inoculation; carpet and glass were the only nonvegetative coupons. Also, in several instances, an organism did not grow on the test material; the test was performed using nonvegetative coupons. For nonvegetative coupons, the test fungus was inoculated to the surface of the materials following the same procedure as previously described for vegetative coupons. The goal was to load each of the individual coupons with 10^6 to 10^7 CFU/coupon. The number of fungal spores on the coupons was enumerated by replicate plating of the positive control coupons (MMBD SOP #009)..

The allergen and mycotoxin preparations were also nonvegetative coupons. For allergen and mycotoxin, pure *Alt. alternata* allergen rAlt a1 (INDOOR Biotechnologies, Ltd., Charlottesville, VA) and Aflatoxin B1 (Romer Labs, Union, MO) were used. The coupons were inoculated by pipet onto the surface of the coupon and allowed to dry in the biosafety cabinet. The amount of allergen and mycotoxin inoculated onto the coupons was determined by replicate analysis of the positive control coupons. Also, rAlt a1 from *A.alternata* vegetative coupons and Asp f1 from *A.fumigatus* vegetative coupons were used for the allergen tests.

- *Organisms*. The organisms selected for this study are usually identified among the mycoflora of mold-contaminated indoor environments. The following mold spores are maintained in the RTI International (Research Triangle Park, NC) (RTI) collection: *Aspergillus versicolor* (RTI 3843), *Aspergillus fumigatus* (RTI 3749), and *Alternaria alternata* (RTI 3211). *Stachybotrys chartarum* (EPA 63-07) was obtained from USEPA; *Chaetomium globosum* (ATCC 34507) was purchased from the American Type Culture Collection (ATCC, Manassas, VA). Spores were inoculated onto each coupon at a concentration of 10^6 – 10^7 spores/coupon. The coupon preparation and the inoculation procedure were performed in accordance with the American Society for Testing and Materials (ASTM) guidelines D 6329-98: “Standard Guide for developing methodology for evaluating the ability of indoor materials to support microbial growth using static environmental chambers.” (ASTM, 1998)

Transportation of coupons. The ClO_2 fumigation of all coupons was performed at the USEPA laboratory in Research Triangle Park, NC. When a set of coupons was ready for the fumigation experiment, the coupons were packaged in Tyvek® envelopes under sterile conditions and transported to the USEPA. Following the fumigation experiment, the set of coupons was returned to RTI for analysis. Chain of custody forms were completed for each transfer step. The coupons were transported under ambient conditions over a distance of about three miles.

Coupon analysis. For CFU determinations, each material coupon was placed in a separate container, suspended in sterile phosphate buffered saline (PBS) containing Tween 80 and shaken for at least thirty minutes. All necessary dilutions were made using the same buffer. Aliquots of the suspension were plated on fungal culture media and incubated for the optimal time and temperature for the test organism. CFU were counted and calculated for each coupon per contractor laboratory standard operating procedures.

Mycotoxin analysis was performed using AgraQuant Aflatoxin (4-40 ppb) test kit from Romer Labs (Union, MO). The coupons were extracted using 70% methanol. The extract or aflatoxin test kit standard was mixed with conjugate in individual dilution wells, and then 100 µL from each dilution well was transferred to an antibody-coated microwell. After incubation at room temperature, the plate was washed, enzyme substrate added, and the plate was reincubated. Stop solution was added, and the intensity of the resulting yellow color was measured optically with a microplate reader (Molecular Devices Emax, Sunnyvale, CA) at a wavelength of 450/650 nm. The chromogenic color change is inversely proportional to the concentration of the toxin in the sample.

Coupons for allergen analysis were extracted into phosphate buffer containing Tween 20. A commercially available ELISA kit by INDOOR Biotechnologies (Charlottesville, VA) was used for analysis of rAlt a1 (Alt. a., allergen 1). Optical density values of both treated and control coupons were plotted against the optical density values of the allergen standard to determine concentration. In addition to the purified rAlt a1 allergen coupons, the coupons prepared for the CFU analyses of *Alternaria alternata* and *Aspergillus fumigatus* were also analyzed for allergen. A portion of the extract from the CFU coupons was removed and bead beaten to break up the spores and/or hyphae present. The antigen present on the *Alternaria alternata* CFU coupons was also quantified with the rAlt a1 ELISA kit. The antigen present on the *Aspergillus fumigatus* CFU coupons was quantified with the Asp f1 ELISA kit.

Calculation of Efficacy. The viability of the organisms was quantified using the ASTM guidelines D 6329-98. The effectiveness of the ClO_2 in inactivation of the culturable test organisms (culturability assay) was quantified by calculating the log change in CFU. First, the $\log_{10}$ CFU per coupon was determined. Next, the average and standard deviation of either the replicate unexposed positive control coupons or the replicate inoculated exposed coupons were calculated. Finally, the log change was calculated as follows:

$$\log_{10} \text{change} = \log_{10} CFU_C - \log_{10} CFU_E \qquad \text{(Eq. 1)}$$

where:

$\log_{10} CFU_C$ = mean $\log_{10}$ CFU of positive control coupons
$\log_{10} CFU_E$ = mean $\log_{10}$ CFU of exposed coupons.

The uncertainty of the efficacy was calculated using the standard deviations from both the exposed and positive control coupons to determine the combined standard error of the difference for each test.

To quantify the effectiveness of the ClO_2 mycotoxins and allergens (mycotoxin and allergen assays), the log change in ng of mycotoxin or allergen was calculated. The ng per coupon of mycotoxin or allergen of either the replicate inoculated unexposed positive control coupons or the replicate inoculated exposed coupons was averaged, and the standard deviation was calculated. The log change was calculated as follows:

$$\log_{10} \text{ng change} = \log_{10} ng_C - \log_{10} ng_E \quad \text{(Eq. 2)}$$

where:

$\log_{10} ng_C$ = mean ng of mycotoxin or allergen of positive control coupons
$\log_{10} ng_E$ = mean ng of mycotoxin or allergen on exposed coupons

The uncertainty of the efficacy was calculated using the standard deviations from both the exposed and positive control coupons to determine the combined standard error of the difference for each test.

Laboratory fumigation procedures: The fumigations were performed at the USEPA laboratory in Research Triangle Park, NC. The fumigation procedure was designed to simulate the conditions used in the field mold remediation process and to provide additional information to assess the efficacy at different CTs. At the time when the application for FIFRA 24 (c) registration was submitted, there were limited data on the efficacy of ClO_2 for mold and related compounds (USEPA, 2006). As a result, the registration label conditions are based on the same 9000 ppmv-hr CT levels used for remediation of *B. anthracis (B.a.)* spores. In the response to the 2001 anthrax remediation, the required CT was achieved by operating at a minimum of 750 ppm for 12 hr. However, for mold remediation, the CT was achieved by operating at a minimum of 3000 ppm for three hr (USEPA, 2012). In addition, the label requires a minimum temperature of 75 °F and 75% RH for the duration of the fumigation. The laboratory fumigation procedure is described below.

Fumigation conditions. The experimental conditions were chosen to span the range of the field conditions used in previous applications. In addition, samples could periodically be removed from the fumigation chamber to allow evaluation of the efficacy of lower CT levels. The baseline condition was 750 ppm for 12 hr. However, intermediate time points were 1.5, 3, 6 and/or 9 hours, corresponding to CT values of 1125, 2250, 4500, 6750 ppmv-hr. The alternative condition was 3000 ppmv for 1 or 3 hours, corresponding to CT values of 3000 and 9000 ppmv-hr. As the experiments progressed, some conditions were adjusted or eliminated for a given organism and/or material to provide better resolution of intermediate CT values. Every organism/material combination was tested at least at 9000 ppmv-hrs CT. Some continuously wetted coupons for pine wood and wallboard were treated at limited CTs (1125 and 2250 ppm-hr). An additional treatment level of 12,000, 18,000, and 24,000 ppmv-hr at 45% RH was used in some tests as included in the test matrix (see Table 1, discussed below).

- *Fumigation chamber:* The fumigation system was initially designed and constructed to allow flexible operation for a variety of fumigation systems. The configuration of the system used in these experiments is shown in Figure 1. The fumigation enclosure is a 300 L (liter) (11 cubic foot (ft^3)) opaque glovebox with provision for periodic removal of a subset of coupons without affecting the fumigation conditions. The coupons, wrapped in Tyvek® envelopes, were placed on racks in the chamber. The system was equipped with fans to ensure that the ClO_2 concentration and humidity were well distributed throughout the chamber. The temperature and RH in the chamber were monitored by a Vaisala RH/Temperature sensor (Vaisala Model HMD40Y,

Vaisala, Helsinki, Finland) that provided feedback to a digital acquisition system (DAS) (LabView, National Instruments, Austin, Texas). When the RH dropped below the set point, the DAS activated a humidity bottle which provided a saturated warm air stream to maintain the process conditions. The DAS also controlled the heat lamps to maintain the process temperature based on feedback from the Vaisala sensor. A ClorDiSys Solutions, Inc. EMS (ClorDiSys EMS, Lebanon, NJ) stand-alone photometric monitor provided continuous monitoring of the ClO_2 concentration in the chamber and provided feedback to the DAS to maintain the required conditions. Modified Standard Method (MSM) 4500-E samples were taken every 20 minutes during the decontamination phase to confirm the concentration of ClO_2 in the chamber. ClO_2 was provided by a Sabre S07-012 system (Sabre, Albany, NY), which supplies a gaseous ClO_2/air stream to the chamber as needed to maintain the required concentration. The aqueous ClO_2 solution was produced externally by mixing hydrochloric acid (Fisher Scientific, Pittsburgh, PA) and sodium chlorite (SabreChlor, Odessa, TX) in the presence of aqueous sodium hypochlorite (12.5%, UnivarUSA, Raleigh, NC). The Sabre system had an emitter, which stripped the ClO_2 from the liquid into an air stream and delivered the ClO_2 to the chamber. The DAS also provided automatic control of the ClO_2 concentration in the chamber. A modified version of the American Water Works Association (AWWA) standard method SM 4500-E (AWWA, 2005) was used to determine ClO_2 concentrations every 20 minutes to compare with the EMS system and to confirm the concentration of ClO_2 in the test chamber.

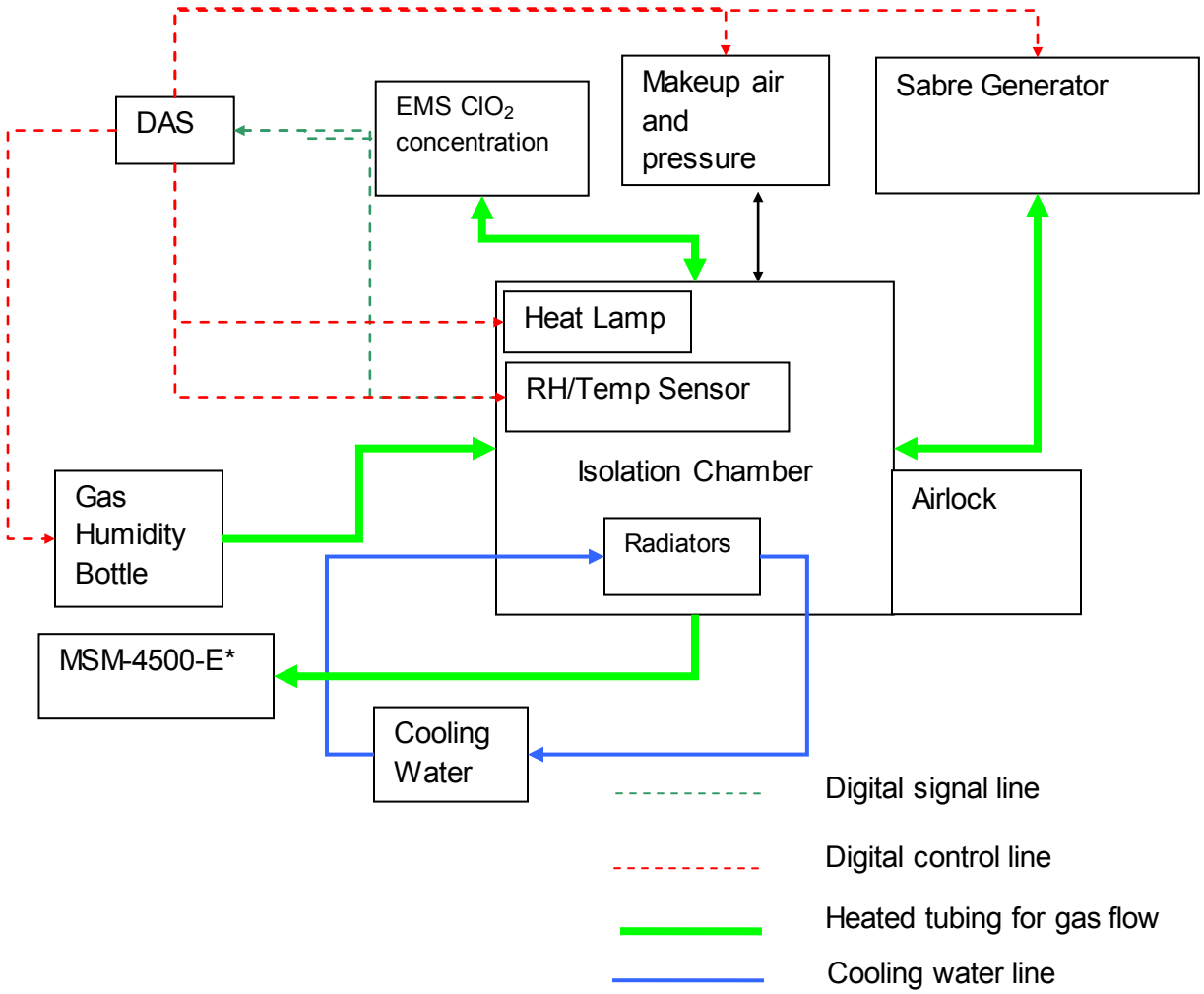

Figure 1 Laboratory Fumigation Schematic
*MSM-4500-E= Modified Standard Method 4500-E

- *Laboratory-scale Fumigation procedure*: The coupons were placed in the chamber and the chamber airlock was sealed. The target temperature and RH for the trial were established and set in the control system. Once the temperature and RH were established, the chamber was charged with ClO_2 to achieve the target concentration. The control system maintained the target ClO_2 concentration, temperature, and RH for the specified time. Time zero is defined as the time at which the target concentration, temperature and RH was achieved in the chamber. Once the desired conditions and time had been achieved, the chamber was aerated until a safe ClO_2 concentration was achieved in the chamber. To achieve the shorter exposure times, the coupons were removed through the airlock (without affecting the chamber conditions) at intermittent time points in the fumigation cycle. The chamber was then opened, and the coupons were removed and placed in the appropriate sample packaging containers. The fumigated coupons were then transferred to RTI for subsequent analysis.

Test Matrix. Table 1 shows the test matrix for ClO_2 fumigation with information on the materials exposed, the contaminant used (mold spores, allergen or mycotoxin), the ClO_2 concentration (ppmv), exposure time, analyses, and the total number of coupons per run. A total of 20 ClO_2 fumigation runs were performed in this study.

Table 1. Test Matrix for Chlorine Dioxide Fumigation[1]

Test ID	Materials	Contamination	Treatment	Time, hours	Analyses	Total Coupons
Run 1	G, L, W, C	*Stachybotrys*	none (pos.control)	0	V(6), M(6)	72
	G, L, W, C	none (blank)	ClO_2 (neg. control)	6, 12	V(1), M(1)	24
	G, L, W, C	*Stachybotrys*	750 ppmv ClO_2	6, 12	V(6), M(6)	144
Run 2	W	*Chaetomium*	none (pos.control)	0	V(6)	6
	W	none (blank)	ClO_2 (neg. control)	3, 6, 9, 12	V(1)	4
	W	*Chaetomium*	750 ppmv ClO_2	3, 6, 9, 12	V(6)	24
Run 3	G, L,C	*Chaetomium*	none (pos.control)	0	V(6)	24
	G, L C	none (blank)	ClO_2 (neg. control)	3, 12	V(1)	8
	G, L, C	*Chaetomium*	750 ppmv ClO_2	3, 12	V(6)	48
Run 4	G, L, W, C	mycotoxin (C1)	none (pos.control)	0	M(6)	24
	G, L, W, C	none (blank)	ClO_2 (neg. control)	6, 9, 12	M(1)	12
	G, L, W, C	mycotoxin (C1)	750 ppmv ClO_2	6, 9, 12	M(6)	72
Run 5	G, L, W, C	rAlt a1extract	none (pos.control)	0	A(6)	24
	G, L, W, C	none (blank)	ClO_2 (neg. control)	6, 9, 12	A(6)	12
	G, L, W, C	rAlt a1extract	750 ppmv ClO_2	6, 9, 12	A(6)	72
Run 6	G, L, W, C	*Aspergillus versicolor*	none (pos.control)	0	V(6), M(6), A(6)	72
	G, L, W, C	none (blank)	ClO_2 (neg. control)	6, 12	V(1), M(1), A(1)	24
	G, L, W, C	*A.versicolor*	750 ppmv ClO_2	6, 12	V(6), M(6), A(6)	144
Run 7	G, L, W, C	*Alternaria*	none (pos.control)	0	V(6), A(6)	48
	G, L, W, C	none (blank)	ClO_2 (neg. control)	6, 12	V(1), A(1)	16
	G, L, W, C	*Alternaria*	750 ppmv ClO_2	6, 12	V(6), A(6)	96
Run 8	G, L, W, C	mycotoxin (C2)	none (pos.control)	0	M(6)	24
	G, L, W, C	none (blank)	3000 ppmv ClO_2	1 (L&W), 3 (all)	M(1)	6
	G, L, W, C	mycotoxin (C2)	3000 ppmv ClO_2	1 (L&W), 3 (all)	M(6)	36
Run 9	G, L, W, C	rAlt a1extract (C2)	none (pos.control)	0	A(6)	24
	G, L, W, C	none (blank)	3000 ppmv ClO_2	1 (L&W), 3 (all)	A(6)	6
	G, L, W, C	rAlt a1extract (C2)	3000 ppmv ClO_2	1 (L&W), 3 (all)	A(6)	36
Run 10	G, L, W, C	*Chaetomium*	none (pos.control)	0	V(6)	24
	G, L, W, C	none (blank)	ClO_2 (neg. control)	1 (L&W), 3 (all)	V(1)	6
	G, L, W, C	*Chaetomium*	3000 ppmv ClO_2	1 (L&W), 3 (all)	V(6)	36
Run 11	G, L, W, C	*Aspergillus fumigatus*	none (pos.control)	0	V(6), M(6), A(6)	72

Test ID	Materials	Contamination	Treatment	Time, hours	Analyses	Total Coupons
	G, L, W, C	none (blank)	ClO_2 (neg. control)	3	V(1), M(1), A(1)	12
	G, L, W, C	*A. fumigatus*	3000 ppmv ClO_2	3	V(6), M(6), A(6)	72
Run 12	G, L, W, C	*Stachybotrys*	none (pos.control)	0	V(6), M(6)	48
	G, L, W, C	none (blank)	ClO_2 (neg. control)	1 (L&W), 3 (all)	V(1), M(1)	12
	G, L, W, C	*Stachybotrys*	3000 ppmv ClO_2	1 (L&W), 3 (all)	V(6), M(6)	72
Run 13	G, L, W, C	*Alternaria*	none (pos.control)	0	V(6), A(6)	48
	G, L, W, C	none (blank)	ClO_2 (neg. control)	3	V(1), A(1)	8
	G, L, W, C	*Alternaria*	3000 ppmv ClO_2	3	V(6), A(6)	48
Run 14	L, W, WW, C	*Chaetomium*	none (pos.control)	0	V(6)	24
	L, W, WW, C	none (blank)	ClO_2 (neg. control)	1.5, 3	V(1)	8
	L, W, WW, C	*Chaetomium*	750 ppmv ClO_2	1.5, 3	V(6)	48
Run 15	Lw, Ww, WWw, Cw	*Chaetomium*	none (pos.control)	0	V(6)	24
	Lw, Ww, WWw, Cw	none (blank)	ClO_2 (neg. control)	1.5, 3	V(1)	8
	Lw, Ww, WWw, Cw	*Chaetomium*	750 ppmv ClO_2	1.5, 3	V(6)	48
Run 16	Lw, Ww, WWw, Cw	*A. fumigatus*	none (pos.control)	0	V(6), A(6)	48
	Lw, Ww, WWw, Cw	none (blank)	ClO_2 (neg. control)	1.5, 3	V(1), A(1)	16
	Lw, Ww, WWw, Cw	*A. fumigatus*	750 ppmv ClO_2	1.5, 3	V(6), A(6)	96
Run 17	L, W, WW, C	Aflatoxin (C1)	none (pos.control)	0	M(6)	24
	L, W, WW, C	none (blank)	ClO_2 (neg. control)	1.5, 3	M(1)	8
	L, W, WW, C	Aflatoxin (C1)	750 ppmv ClO_2	1.5, 3	M(6)	48
Run 18	L, W, WW, C	*A. fumigatus*	none (pos.control)	0	V(6), A(6)	48
	L, W, WW, C	none (blank)	ClO_2 (neg. control)	1.5, 3	V(1), A(1)	16
	L, W, WW, C	*A. fumigatus*	750 ppmv ClO_2	1.5, 3	V(6), A(6)	96
Run 19	L, W, WW, C	*Chaetomium*	none (pos.control)	0	V(6)	24
	L, W, WW, C	none (blank)	ClO_2 (neg. control)	3, 6	V(1)	8
	L, W, WW, C	*Chaetomium*	3000 ppmv ClO_2 45% RH	3, 6	V(6)	48
Run 20	L, W, WW, C	*Chaetomium*	none (pos.control)	0	V(6)	24
	L, W, WW, C	none (blank)	ClO_2 (neg. control)	3, 6	V(1)	8
	L, W, WW, C	*Chaetomium*	4000 ppmv ClO_2 45% RH	3, 6	V(6)	48

[1] Yellow highlight indicates runs where mycotoxin data were not reported. Components of the building materials interfered with mycotoxin assays.

Key: Materials: G = glass; L = latex-painted wallboard; W = unpainted pine wood; C = industrial carpet; WW = wallpapered latex-painted wallboard; C = ceiling tile (grown at 100% RH, but dry by the time they were treated).

Lw, Ww, WWw, Cw (Same materials as above but grown at 100% RH with wetting prior to inoculation, wetting maintained during treatment and return for analysis).

Analysis: V = viability; A = antigen; M = mycotoxin. Numbers in parentheses indicate the number of coupons used for that particular test.

3. RESULTS AND DISCUSSION

The objective of this study was to determine the biocidal efficacy of ClO_2 fumigation against a variety of fungi (molds) and their nonviable components. A series of tests was performed using either glass slides or one of five building materials as the test coupons. These building materials were latex-painted gypsum wallboard, wallpapered gypsum wallboard, bare structural pine wood, industrial-grade carpet, and ceiling tile representing real-life structural building materials (the painted walls, the flooring, and ceiling). These materials permitted the evaluation of the efficacy of using ClO_2 gas as a decontamination approach in the built environment as realistically as possible in a controlled laboratory setting. Glass was chosen as a control material that would give the best-case inactivation results with the least amount of variability caused by confounding factors. The building materials have the potential to impede the inactivation of biological contaminants by ClO_2, whereas glass is a smooth hard surface and is, thus, less likely to interfere.

At present, there are no standard procedures for the evaluation of biocides used for remediation of mold-contaminated buildings. The methodology used in this study was developed according to ASTM D 6329-98. To evaluate the effectiveness of ClO_2 gas to inactivate the test organisms, the CFU of exposed test control coupons were compared to CFU of unexposed positive control coupons. For the calculations, CFU values were converted to their logarithmic (base 10) form. A key issue in evaluating the efficacy of ClO_2 fumigation was to determine the acceptable number of CFU remaining after treatment. A panel of EPA ORD researchers and Program Office experts from OPP and the Office of Radiation and Indoor Air (ORIA) agreed that the target reduction for mold was 4 $\log_{10}$ (99.99%). Therefore, the challenge level (CFU concentration per coupon prior to ClO_2 exposure) was based on being able to quantify a 4 $\log_{10}$ reduction (change) in CFU after ClO_2 exposure. The USEPA previously reported a similar methodology for the evaluation of the antimicrobial efficacy of ozone (Menetrez et al., 2009).

Tables 2 through 6 show the results for each of the materials tested. All of the tables have the same format and include results from both vegetative and nonvegetative coupons. The first column represents the ClO_2 concentration (ppmv). The second column represents the exposure time (hr). The third column represents the total exposure CT (ppmv-hr). The fourth column represents the averaged $\log_{10}$ CFU results with standard deviations of unexposed positive controls, and the fifth column represents the averaged $\log_{10}$ CFU results with standard deviations of exposed test coupons. The sixth column represents the CFU $\log_{10}$ change as shown in equation 1 (materials and methods). The seventh column represents the % of inactivation efficiency, calculated with Equation 3. Table 2 shows the results for the latex-painted and the wallpapered gypsum wallboard. Table 3 shows the results for ceiling tile. Table 4 shows the results for unpainted pine wood. Table 5 shows the results for carpet. Table 6 shows the results for glass. When 0 (zero) CFU were detected on all the replicate plates from one coupon, 0.5 was used as the number of CFU detected and used to calculate of the number of CFU on a coupon. When that number of CFU on a coupon was transformed to CFU $\log_{10}$, the ≤ symbol was used in the tables to indicate that no actual CFU were detected. Consequently, when the ≤

CFU log_{10} was used in the calculation of the log_{10} change, the ≥ symbol is used to express the log_{10} change.

$$\% \text{ inactivation efficiency} = \frac{\text{Conc. Control} - \text{Conc. Exposed}}{\text{Conc. Control}} \times 100 \qquad \text{(Eq. 3)}$$

Table 2. Summary of Results for Latex-Painted Wallboard and Wallpapered Latex-Painted Wallboard (all vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev.	% Inactivation Efficacy
S. chartarum						
Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
3000	1	3000	6.75 ± 0.17	≤ 2.00 ± 0.00	≥ 4.75 ± 0.12	≥ 99.99
750	6	4500	6.78 ± 0.05	≤ 2.00 ± 0.00	≥ 4.78 ± 0.03	≥ 99.99
750	12	9000	6.78 ± 0.05	≤ 2.00 ± 0.00	≥ 4.78 ± 0.03	≥ 99.99
3000	3	9000	6.75 ± 0.17	≤ 2.00 ± 0.00	≥ 4.75 ± 0.12	≥ 99.99
C. globosum						
Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	5.82 ± 0.21	2.55 ± 0.64	3.27 ± 0.48	81.74
750	3	2250	5.82 ± 0.21	1.58 ± 0.31	4.24 ± 0.28	99.99
750	3	2250	5.67 ± 0.62	3.42 ± 0.74	2.25 ± 0.68	56.24
3000	1	3000	5.90 ± 0.22	2.46 ± 0.91	3.44 ± 0.67	85.99
750	12	9000	5.67 ± 0.62	1.73 ± 0.67	3.94 ± 0.64	98.49
3000	3	9000	5.80 ± 0.19	2.99 ± 0.59	4.55 ± 0.18	99.99
Latex-Painted Wallboard Coupons; 45 % RH ClO_2 Exposure						
3000	3	9000	5.80 ± 0.19	2.99 ± 0.59	2.81 ± 0.44	70.24
4000	3	12000	5.78 ± 0.16	2.91 ± 0.56	2.87 ± 0.41	71.74
3000	6	18000	5.80 ± 0.19	2.65 ± 0.48	3.14 ± 0.36	78.42
4000	6	24000	5.78 ± 0.16	1.78 ± 0.79	3.99 ± 0.57	99.74
Latex-Painted Wallboard Wetted Coupons* ; 75% RH ClO_2 Exposure						
750	1.5	1125	5.66 ± 0.17	2.16 ± 0.34	3.50 ± 0.27	87.49
750	3	2250	5.66 ± 0.17	2.23 ± 0.65	3.43 ± 0.47	85.66
Wallpapered Latex-Painted Wallboard Coupons; 75% RH ClO2 Exposure						
750	1.5	1125	5.53 ± 0.40	3.46 ± 0.87	2.07 ± 0.68	51.74
750	3	2250	5.53 ± 0.40	3.39 ± 0.72	2.14 ± 0.58	53.49
Wallpapered Latex-Painted Wallboard Coupons; 45 % RH ClO_2 Exposure						
3000	3	9000	5.71 ± 0.35	2.63 ± 0.41	3.08 ± 0.38	76.99
4000	3	12000	5.97 ± 0.18	1.73 ± 0.44	4.25 ± 0.34	99.99

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev.	% Inactivation Efficacy
3000	6	18000	5.71 ± 0.35	2.03 ± 0.16	3.69 ± 0.27	92.24
4000	6	24000	5.97 ± 0.18	1.61 ± 0.50	4.36 ± 0.36	99.99
Wallpapered Latex-Painted Wallboard Wetted Coupons ; 75% RH ClO_2 Exposure						
750	1.5	1125	6.28 ± 0.18	4.84 ± 0.31	1.44 ± 0.25	35.99
750	3	2250	6.28 ± 0.18	4.21 ± 0.84	2.07 ± 0.61	51.74
A. versicolor						
Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
750	6	4500	6.67 ± 0.02	1.93 ± 0.12	4.75 ± 0.09	99.99
750	12	9000	6.67 ± 0.02	≤ 1.88 ± 0.00	≥ 4.80 ± 0.02	≥ 99.99
A. fumigatus						
Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	7.58 ± 0.17	3.91 ± 1.41	3.67 ± 1.01	91.74
750	3	2250	7.58 ± 0.17	2.09 ± 0.40	5.49 ± 0.31	99.99
3000	3	9000	7.46 ± 0.15	2.15 ± 0.25	5.31 ± 0.21	99.99
Latex-Painted Wallboard Wetted Coupons ; 75% RH ClO_2 Exposure						
750	1.5	1125	7.17 ± 0.48	7.06 ± 0.09	0.11 ± 0.35	2.75
750	3	2250	7.17 ± 0.48	7.05 ± 0.19	0.12 ± 0.36	2.99
Wallpapered Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	7.52 ± 0.22	6.83 ± 0.53	0.69 ± 0.41	17.24
750	3	2250	7.52 ± 0.22	6.69 ± 0.68	0.83 ± 0.51	20.74
Wallpapered Latex-Painted Wallboard Wetted Coupons ; 75% RH ClO_2 Exposure						
750	1.5	1125	7.79 ± 0.29	7.12 ± 0.18	0.67 ± 0.24	16.74
750	3	2250	7.79 ± 0.29	7.06 ± 0.05	0.73 ± 0.21	18.24
A. alternata						
Latex-Painted Wallboard Coupons; 75% RH ClO_2 Exposure						
750	6	4500	3.90 ± 0.84	≤ 0.88 ± 0.00	≥ 3.03 ± 0.60	≥ 99.99
750	12	9000	3.90 ± 0.84	≤ 0.88 ± 0.00	≥ 3.03 ± 0.60	≥ 99.99
3000	3	9000	4.23 ± 0.30	≤ 0.88 ± 0.00	≥ 3.36 ± 0.21	≥ 99.99

*Wetted-vegetative coupons were coupons wetted prior to inoculation and maintained w etted during treatment.
Brow n-shaded area - w etted-vegetative coupons.
Pink-shaded area- 45% RH ClO_2 exposure.

Table 3. Summary of Results for Ceiling Tile (all vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure-CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev.	% Inactivation Efficacy
C. globosum						
Ceiling Tile Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	7.32 ± 0.21	5.72 ± 0.55	1.59 ± 0.41	39.74
750	3	2250	7.32 ± 0.21	3.3 ± 0.29	3.99 ± 0.26	99.74
Ceiling Tile Coupons; 45 % RH ClO_2 Exposure						
3000	3	9000	7.29 ± 0.18	4.67 ± 0.26	2.62 ± 0.22	65.49
4000	3	12000	6.85 ± 0.09	3.99 ± 0.71	2.86 ± 0.51	71.49
3000	6	18000	7.29 ± 0.18	4.14 ± 0.16	3.15 ± 0.17	78.74
4000	6	24000	6.85 ± 0.09	3.26 ± 0.18	3.60 ± 0.14	89.99
Ceiling Tile Wetted Coupons* ; 75% RH ClO_2 Exposure						
750	1.5	1125	7.13 ± 0.23	6.15 ± 0.39	0.98 ± 0.32	24.49
750	3	2250	7.13 ± 0.23	5.76 ± 0.46	1.36 ± 0.37	33.99
A. fumigatus						
Ceiling Tile Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	8.00 ± 0.40	2.03 ± 0.53	5.97 ± 0.47	99.99
750	3	2250	8.00 ± 0.40	≤ 1.88 ± 0.00	≥ 6.12 ± 0.28	≥ 99.99
Ceiling Tile Wetted Coupons* ; 75% RH ClO_2 Exposure						
750	1.5	1125	9.19 ± 0.12	8.34 ± 0.23	0.85 ± 0.18	21.24
750	3	2250	9.19 ± 0.12	8.02 ± 0.23	1.17 ± 0.19	29.24

*Wetted-vegetative coupons were coupons wetted prior to inoculation and maintained wetted during treatment.
Brown-shaded area - wetted-vegetative coupons.
Pink-shaded area- 45% RH ClO_2 exposure.

Table 4. Summary of Results for Unpainted Pine Wood

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev.	% Inactivation Efficacy
S. chartarum						
Unpainted Pine Wood non-Vegetative Coupons; 75% RH ClO_2 Exposure						
3000	1	3000	7.63 ± 0.13	2.05 ± 0.12	5.58 ± 0.13	99.99
750	6	4500	7.24 ± 0.17	≤ 2.00 ± 0.00	≥ 5.24 ± 0.12	≥ 99.99
750	12	9000	7.24 ± 0.17	≤ 2.00 ± 0.00	≥ 5.24 ± 0.12	≥ 99.99
3000	3	9000	7.63 ± 0.13	≤ 2.00 ± 0.00	≥ 5.63 ± 0.09	≥ 99.99
C. globosum						
Unpainted Pine Wood Vegetative Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	4.53 ± 0.23	1.07 ± 0.68	3.46 ± 0.51	86.49
750	3	2250	4.53 ± 0.23	1.02 ± 0.62	3.52 ± 0.47	87.99
750	3	2250	5.39 ± 0.26	0.93 ± 0.12	4.46 ± 0.20	99.99
3000	1	3000	5.20 ± 0.14	0.98 ± 0.25	4.22 ± 0.20	99.99
750	6	4500	5.39 ± 0.26	1.03 ± 0.35	4.36 ± 0.31	99.99
750	9	6750	5.39 ± 0.26	≤ 0.88 ± 0.00	≥ 4.51 ± 0.18	≥ 99.99
750	12	9000	5.39 ± 0.26	≤ 0.88 ± 0.00	≥ 4.51 ± 0.18	≥ 99.99
3000	3	9000	5.20 ± 0.14	≤ 0.88 ± 0.00	≥ 4.33 ± 0.10	≥ 99.99
Unpainted Pine Wood Vegetative Coupons; 45 % RH ClO_2 Exposure						
3000	3	9000	4.72 ± 0.18	≤ 1.57 ± 0.00	≥ 3.15 ± 0.76	≥ 99.99
4000	3	12000	4.57 ± 0.44	0.61 ± 0.98	3.96 ± 0.76	98.99
3000	6	18000	4.72 ± 0.18	≤ 1.57 ± 0.00	≥ 3.15 ± 0.76	≥ 99.99
4000	6	24000	4.57 ± 0.44	0.61 ± 0.98	3.96 ± 0.76	98.99
Unpainted Pine Wood Vegetative Wetted Coupons* ; 75% RH ClO_2 Exposure						
750	1.5	1125	5.34 ± 0.29	1.76 ± 0.29	3.58 ± 0.75	89.49
750	3	2250	5.34 ± 0.29	≤ 1.18 ± 0.00	4.16 ± 0.20	99.99
A. versicolor						
Unpainted Pine Wood Vegetative Coupons; 75% RH ClO_2 Exposure						
750	6	4500	6.23 ± 0.12	≤ 1.88 ± 0.00	≥ 4.35 ± 0.08	≥ 99.99
750	12	9000	6.23 ± 0.12	≤ 1.88 ± 0.00	≥ 4.35 ± 0.08	≥ 99.99
A. fumigatus						
Unpainted Pine Wood Vegetative Coupons; 75% RH ClO_2 Exposure						
750	1.5	1125	4.71 ± 0.45	2.00 ± 0.32	2.71 ±0.39	67.74
750	3	2250	4.71 ± 0.45	2.31 ± 0.92	2.40 ± 0.73	59.99
3000	3	9000	6.56 ± 0.32	≤ 2.00 ± 0.00	≥ 4.56 ± 0.23	≥ 99.99

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev.	% Inactivation Efficacy
Unpainted Pine Wood Vegetative Wetted Coupons ; 75% RH ClO_2 Exposure						
750	1.5	1125	7.58 ± 0.22	2.30 ± 1.53	5.28 ± 1.10	99.99
750	3	2250	7.58 ± 0.22	1.56 ± 0.66	6.02 ± 0.49	99.99
A. alternata						
Unpainted Pine Wood non-Vegetative coupons; 75% RH ClO_2 Exposure						
750	6	4500	6.29 ± 0.08	≤ 1.88 ± 0.00	≥ 4.42 ± 0.06	≥ 99.99
750	12	9000	6.29 ± 0.08	≤ 1.88 ± 0.00	≥ 4.42 ± 0.06	≥ 99.99
3000	3	9000	7.01 ± 0.06	≤ 1.88 ± 0.00	≥ 5.13 ± 0.04	≥ 99.99

*Wetted vegetative coupons were coupons wetted prior to inoculation and maintained wetted during treatment.
Brown-shaded area - wetted vegetative coupons.
Pink-shaded area- 45% RH ClO_2 exposure.

Table 5. Summary of Results for Carpet (all non-vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev	% Inactivation Efficacy
75% RH ClO_2 Exposure						
S. chartarum						
750	6	4500	7.15 ± 0.12	2.32 ± 0.41	4.83 ± 0.30	99.99
750	12	9000	7.15 ± 0.12	2.04 ± 0.11	5.11 ± 0.12	99.99
3000	3	9000	7.46 ± 0.14	≤ 2.00 ± 0.00	≥ 5.46 ± 0.10	≥ 99.99
C. globosum						
750	3	2250	7.19 ± 0.07	3.70 ± 0.20	3.48 ± 0.15	86.99
750	12	9000	7.19 ± 0.07	2.18 ± 0.27	≥ 5.01 ± 0.20	≥ 99.99
3000	3	9000	7.64 ± 0.08	2.62 ± 0.55	5.03 ± 0.39	99.99
A. versicolor						
750	6	4500	6.94 ± 0.20	≤ 1.88 ± 0.00	≥ 5.07 ± 0.14	≥ 99.99
750	12	9000	6.94 ± 0.20	≤ 1.88 ± 0.00	≥ 5.07 ± 0.14	≥ 99.99
A. fumigatus						
3000	3	9000	7.16 ± 0.17	≤ 2.00 ± 0.00	≥ 5.16 ± 0.12	≥ 99.99
A. alternata						
750	6	4500	5.93 ± 0.20	≤ 1.88 ± 0.00	≥ 4.05 ± 0.14	≥ 99.99
750	12	9000	5.93 ± 0.20	≤ 1.88 ± 0.00	≥ 4.05 ± 0.14	≥ 99.99
3000	3	9000	6.91 ± 0.44	≤ 1.88 ± 0.00	≥ 5.04 ± 0.31	≥ 99.99

Table 6. Summary of Results for Glass (all non-vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (CFU log_{10}) Mean ± St. Dev.	Exposed Coupons (CFU log_{10}) Mean ± St. Dev.	Log Change (CFU log_{10}) Mean ± St. Dev	% Inactivation Efficacy
75% RH ClO_2 Exposure						
S. chartarum						
750	6	4500	7.46 ± 0.01	≤ 2.00 ± 0.00	≥ 5.46 ± 0.01	≥ 99.99
750	12	9000	7.46 ± 0.01	≤ 2.00 ± 0.00	≥ 5.46 ± 0.01	≥ 99.99
3000	3	9000	7.53 ± 0.17	≤ 2.00 ± 0.00	≥ 5.53 ± 0.12	≥ 99.99
C. globosum						
750	3	2250	7.33 ± 0.07	4.55 ± 0.36	2.78 ± 0.26	69.49
750	12	9000	7.33 ± 0.07	3.10 ± 0.87	4.24 ± 0.62	99.99
3000	3	9000	7.75 ± 0.07	2.91 ± 0.79	4.83 ± 0.56	99.99
A. versicolor						
750	3	4500	6.08 ± 0.13	≤ 1.88 ± 0.00	≥ 4.21 ± 0.09	≥ 99.99
750	12	9000	6.08 ± 0.13	≤ 1.88 ± 0.00	≥ 4.21 ± 0.09	≥ 99.99
A. fumigatus						
3000	3	9000	6.91 ± 0.22	≤ 2.00 ± 0.00	≥ 4.91 ± 0.15	≥ 99.99
A. alternata						
750	6	4500	5.49 ± 0.57	≤ 1.88 ± 0.00	≥ 3.61 ± 0.40	≥ 99.99
750	12	9000	5.49 ± 0.57	≤ 1.88 ± 0.00	≥ 3.61 ± 0.40	≥ 99.99
3000	3	9000	6.98 ± 0.11	≤ 1.88 ± 0.00	≥ 5.10 ± 0.08	≥ 99.99

A ≥4 log reduction in CFU was seen on latex-painted gypsum wallboard, unpainted pine wood, industrial carpet, and glass for *A. alternata* and *S. chartarum* at CTs of 3,000 or 4,500 ppm-hr. *A. versicolor* was tested only at very high CTs and showed a ≥4 log reduction. However, *A. fumigatus* was tested at much lower CTs, and the level of kill was highly variable and dependent upon building material type. The results of the *C. globosum* testing also show a material dependence for the efficacy of kill.

Most of the ClO_2 fumigations at 75% RH were performed using standard dry materials (coupons) prior to inoculation with mold spores. However, some ClO_2 fumigations at 75% RH using wetted coupons prior to inoculation were tested with *C. globosum* and *A. fumigatus*. The level of kill for *C. globosum* on the wetted coupons was similar to that attained for the dry coupons except for ceiling tile. A ≥4 log_{10} reduction was observed for dry ceiling tile at a CT of 2250 ppmv-hr. No significant inactivation was observed on the wetted ceiling tile coupons exposed to the same CT. Results were highly variable for *A. fumigatus*. For wetted wood coupons, a ≥4 LR was observed at a CT of 1125 ppmv-hr. All the other materials tested showed a lower level of kill on the wetted coupons.

A series of ClO_2 fumigations was performed at 45% RH at high CTs. *C. globosum* was the only organism exposed under these conditions. A ≥4 log_{10} reduction was observed for latex-painted

wallboard, wallpapered latex-painted wallboard and unpainted pine wood at 12000 or 24000 ppmv-hr. A lower kill was observed on ceiling tile exposed to the same conditions.

In some cases, although a ≥4 $\log_{10}$ reduction in CFU was attained, there were still viable spores on the coupons (data not shown). These spores may have the ability to germinate should the environmental conditions become or remain favorable. The germination potential was not evaluated.

To evaluate the effectiveness of ClO_2 gas to inactivate mold components, the mass (nanograms, ng) of allergen or mycotoxin on the exposed test coupons was compared to ng of allergen or mycotoxin of unexposed positive control coupons. The percent change was calculated using Equation 4:

$$\% \text{ inactivation allergen (mycotoxin)} = \frac{\text{Conc. Control} - \text{Conc. Exposed}}{\text{Conc. Control}} \times 100 \qquad \text{(Eq. 4)}$$

Using this formula, the results were analyzed using the averaged ng concentration of allergens (mycotoxins) for both unexposed and exposed coupons and excluding negative $\log_{10}$ values. All the tables have the same format. The first column represents the ClO_2 concentration (ppmv). The second column represents the exposure time (hr). The third column represents the total exposure CT, in ppmv-hours. The fourth column represents the averaged ng concentrations of unexposed positive controls and the fifth column represents the averaged ng concentrations of exposed coupons. The sixth column represents the % inactivation of allergen (mycotoxin) calculated using equation 4. The $\log_{10}$ change for the allergens and mycotoxins on each of the materials tested was calculated using equation 2 and is shown inTables A1-A4 (Appendix A).

Table 7 shows the results for pure allergen rAlt a1 on each of the materials tested, except for latex-painted wallboard. ClO_2 exposures ≥ 4500 showed a 99% reduction in recovered allergen in all the materials tested. For latex-painted wallboard, results were variable; at CTs of 3000 and 9000 ppmv-hr, a 99% inactivation was attained, and all the other CTs tested showed a lower degree of inactivation. Table 10 shows the results for allergen rAlt a1 on *A. alternata* non-vegetative coupons. All materials tested showed a 97 – 99% reduction in Alt a1 allergen. Table 9 shows the results for allergen Asp f1 on vegetative coupons. Results showed that the highest % of allergen Asp f1 inactivation was attained at 9000 ppmv-hr on latex–painted wallboard followed by *Aspergillus fumigatus* antigen (Asp f1) inactivation of ≥ 85.55 % on unpainted pine wood. Results for all the other materials tested were highly variable, and there was no clear distinction on which CT effectively reduced the allergen concentrations.

Table 8 shows the results for pure aflatoxin. On latex-painted wallboard, the highest % inactivation was observed at a ClO_2 concentration of 750 ppmv for 12 hours (9000 ppmv-hr) of exposure. On carpet, the highest % inactivation was observed at a ClO_2 concentration of 3000 ppmv for three hours (9000 ppmv-hr). On unpainted pine wood, at CTs ≥ 3000 ppmv, a 93 - 94% inactivation was attained. Wallpapered wallboard and ceiling tiles were tested only at CTs of

1125 and 2250 ppmv-hr. On ceiling tiles, a 96% inactivation was attained for both CTs. However, for latex-painted wallpapered wallboard, these CTs were ineffective. On glass, all CTs tested showed an aflatoxin inactivation ≥ 90%. The most effective condition for glass was a ClO_2 concentration of 3000 ppmv for 3 hrs (9000 ppmv-hr) of exposure.

Table 11 shows a summary of the % of inactivation efficacy of CFU on all materials. Only tests with 99.99% of inactivation efficacy are presented in this table.

Table 7. Summary of Results for Pure Allergen rAlt a1 on All Materials (non-vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (ng)	Exposed Coupons (ng)	% Allergen nactivation[a]
Latex-painted Wallboard					
3000	1	3000	912.01	4.57	99.50
750	6	4500	831.76	70.79	91.49
750	9	6750	831.76	79.43	90.45
750	12	9000	831.76	81.28	90.23
3000	3	9000	912.01	10.72	98.83
Unpainted Pine Wood					
3000	1	3000	602.56	74.13	87.70
750	6	4500	707.95	≤ 1.00	≥ 99.86
750	9	6750	707.95	≤ 1.00	≥ 99.86
750	12	9000	707.95	≤ 1.00	≥ 99.86
3000	3	9000	602.56	4.57	99.24
Carpet					
750	6	4500	831.76	2.04	99.75
750	9	6750	831.76	1.32	99.84
750	12	9000	831.76	1.41	99.83
3000	3	9000	933.25	3.09	99.67
Glass					
750	6	4500	776.25	≤ 1.00	≥ 99.87
750	9	6750	776.25	≤ 1.00	≥ 99.87
750	12	9000	776.25	≤ 1.00	≥ 99.87
3000	3	9000	575.44	2.04	99.65

[a], (concentration control coupons - concentration of exposed coupons / concentration of control coupons) * 100.

Table 8. Summary of Results for Pure Aflatoxin on All Materials (non-vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Total Exposure - CT (ppmv-hr)	Control Coupons (ng)	Exposed Coupons (ng)	% aflatoxin inactivation[a]
Latex-painted Wallboard					
750	1.5	1125	89.13	24.55	72.46
750	3	2250	89.13	11.75	86.82
3000	1	3000	66.07	14.79	77.61
750	6	4500	104.71	6.92	93.39
750	9	6750	104.71	4.47	95.73
750	12	9000	104.71	≤ 3.98	≥ 96.20
3000	3	9000	66.07	4.27	93.54
Wallpapered Latex-painted Wallboard					
750	1.5	1125	70.79	32.36	54.29
750	3	2250	70.79	23.99	66.12
Ceiling tile					
750	1.5	1125	309.03	10.96	96.45
750	3	2250	309.03	10.47	96.61
Unpainted Pine Wood					
750	1.5	1125	46.77	≤ 7.94	≥ 83.02
750	3	2250	46.77	8.13	82.62
3000	1	3000	67.61	≤ 3.98	≥ 94.11
750	6	4500	69.18	4.57	93.39
750	9	6750	69.18	4.47	93.54
750	12	9000	69.18	4.27	93.83
3000	3	9000	67.61	≤ 3.98	≥ 94.11
Carpet					
750	6	4500	51.29	26.30	48.71
750	9	6750	51.29	9.33	81.80
750	12	9000	51.29	6.61	87.12
3000	3	9000	100.00	7.76	92.24
Glass					
750	6	4500	47.86	≤ 3.98	≥ 91.68
750	9	6750	47.86	≤ 3.98	≥ 91.68
750	12	9000	47.86	≤ 3.98	≥ 91.68
3000	3	9000	154.88	5.50	96.45

[a], (concentration control coupons - concentration of exposed coupons / concentration of control coupons) * 100

Table 9. Summary of Results for Asp f 1 Allergen from *Aspergillus fumigatus* CFU Inoculated Positive Controls and Exposed Coupons in All Materials (vegetative coupons)

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons[a] (ng)	Exposed Coupons (ng)	% allergen inactivation[b]
Latex-painted Wallboard					
750	1.5	1125	0.26	0.30	-14.32
750	3	2250	0.26	≤ 0.40	≥ -51.36
3000	3	9000	8.32	≤ 0.40	≥ 95.21
Wallpapered Latex-painted Wallboard					
750	1.5	1125	0.19	0.22	- 17.49
750	3	2250	0.19	0.30	- 58.49
Ceiling Tile					
750	1.5	1125	0.13	0.12	8.80
750	3	2250	0.13	0.32	-139.88
Unpainted Pine Wood					
750	1.5	1125	0.17	0.18	- 2.33
750	3	2250	0.17	0.18	- 2.33
3000	3	9000	2.75	≤ 0.40	≥ 85.55

[a], Asp f 1 allergen data are not shown for carpet and glass because the allergen is a hyphal allergen. Asp f 1is not shown for latex-painted wallboard wetted coupons, wallpapered latex-painted wallboard wetted coupons, ceiling tile wetted coupons and unpainted pine wood wetted coupons because the allergen concentrations in the controls were below the detection limits.

[b], (concentration control coupons - concentration of exposed coupons / concentration of control coupons) * 100.

Table 10. Summary of Results for Alt a 1 Allergen from *Alternaria alternata* CFU Inoculated Positive Controls and Exposed Coupons on All Materials (non-vegetative coupons)[a]

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (ng)	Exposed Coupons (ng)	% allergen inactivation[b]
Unpainted Pine Wood					
750	6	4500	158.49	3.55	97.76
750	12	9000	158.49	2.24	98.59
3000	3	9000	177.83	1.12	99.37
Carpet					
750	6	4500	891.25	3.63	99.59
750	12	9000	891.25	1.55	99.83
3000	3	9000	74.13	1.15	98.45
Glass					
750	6	4500	363.08	3.55	99.02
750	12	9000	363.08	3.72	98.98
3000	3	9000	89.13	≤ 1.00	≥ 98.88

[a], Alt a 1 allergen data are not shown for latex-painted gypsum wallboard due to material assay interference or insufficient spore production on the growing sample.

[b], (concentration control coupons - concentration of exposed coupons / concentration of control coupons) * 100.

Table 11. Percent Inactivation Efficacy of CFU on All Materials

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	*Stachybotrys chartarum*	*Chaetomium globosum*	*Aspergillus*		*Alternaria alternata*
					versicolor	*fumigatus*	
Latex-painted Gypsum Wallboard (all vegetative coupons)							
75% RH ClO_2 Exposure							
750	1.5	1125	—		—		—
750	3	2250	—	99.99	—	99.99	—
750	3	2250	—		—	—	—
3000	1	3000	≥ 99.99		—	—	—
750	6	4500	≥ 99.99	—	99.99	—	≥ 99.99
750	12	9000	≥ 99.99		≥ 99.99	—	≥ 99.99
3000	3	9000	≥ 99.99	99.99	—	99.99	≥ 99.99
45% RH ClO_2 Exposure							
4000	6	24,000	—	99.74	—	—	—
Wallpapered Latex-painted Wallboard (all vegetative coupons)							
45% RH ClO_2 Exposure							
4000	3	12,000	—	99.99	—	—	—
4000	6	24,000	—	99.99	—	—	—
Ceiling Tile (all vegetative coupons)							
75% RH ClO_2 Exposure							
750	1.5	1125	—		—	99.99	—
750	3	2250	—	99.74	—	≥ 99.99	—
Unpainted Pine Wood (*A.versicolor*, *C.globosum*, and *A.fumigatus*: vegetative coupons; *S. chartarum* and *A.alternata*: non-vegetative coupons)							
75% RH ClO_2 Exposure							
750	1.5	1125	—		—		—
750	3	2250	—		—		—
750	3	2250	—	99.99	—	—	—
3000	1	3000	99.99	99.99	—	—	—

ClO_2 Concentration (ppmv)	Exposure Time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	*Stachybotrys chartarum*	*Chaetomium globosum*	*Aspergillus*		*Alternaria alternata*
					versicolor	*fumigatus*	
750	6	4500	≥ 99.99	99.99	≥ 99.99	—	≥ 99.99
750	9	6750	—	≥ 99.99	—	—	—
750	12	9000	≥ 99.99	≥ 99.99	≥ 99.99	—	≥ 99.99
3000	3	9000	≥ 99.99	≥ 99.99	—	≥ 99.99	≥ 99.99
45% RH ClO_2 Exposure							
3000	3	9000	—	≥ 99.99	—	—	—
4000	3	12000		98.99			
3000	6	18,000	—	≥ 99.99	—	—	—
4000	6	24000		98.99			
75% RH ClO_2 Exposure - Wetted Coupons							
750	1.5	1125	—		—	99.99	—
750	3	2250	—	99.99	—	99.99	—
Carpet (all non-vegetative coupons)							
75% RH ClO_2 Exposure							
750	3	2250	—		—	—	—
750	6	4500	99.99	—	≥ 99.99	—	≥ 99.99
750	12	9000	99.99	≥ 99.99	≥ 99.99	—	≥ 99.99
3000	3	9000	≥ 99.99	99.99	—	≥ 99.99	≥ 99.99
Glass (all non-vegetative coupons)							
75% RH ClO_2 Exposure							
750	3	2250	—		—	—	—
750	6	4500	≥ 99.99	—	≥ 99.99	—	≥ 99.99
750	12	9000	≥ 99.99	99.99	≥ 99.99	—	≥ 99.99
3000	3	9000	≥ 99.99	99.99	—	≥ 99.99	≥ 99.99

*, Only tests with 99.99% of inactivation efficacy are presented in this table.

4. CONCLUSIONS AND RECOMMENDATIONS

The efficacy of ClO_2 fumigation for remediation of mold and related allergens and mycotoxins was evaluated for five commonly found mold species on five typical building materials and glass as a standard material for biological testing. The different interactions between the various mold species and the materials resulted in two types of coupons: coupons where the coupons were incubated and the mold grew (vegetative coupons) and coupons where there was no growth and the original spores remained (nonvegetative coupons). The reduction in viable spores was determined by subtracting the average log values of CFU recovered from the treated coupons from the CFU recovered from the positive controls. In the case of *Alternaria*, the pre- and post-inoculated coupons were extracted to quantify the allergen concentration reduction. Coupons were also prepared by inoculation with either a pure allergen and/or mycotoxin. The results obtained lead to the following conclusions:

Mold viability: The hypothesis of the experiment was that ClO_2 fumigation had the potential to achieve a 4 $\log_{10}$ reduction in viable spores on typical building materials. The target concentration to achieve this result was a CT of 9000 ppmv-hr. This CT was achieved by treating either at 750 ppm for 12 hours or at 3000 ppm for 3 hours. However, additional CT values (2250, 4500, 6750 ppmv-hr) were also evaluated to determine efficacy at intermediate time points. A limited set of tests was performed at an alternative fumigation condition of 18,000 or 24,000 ppm-hr at 45% RH. Another limited series of tests was performed on wetted coupons at low CT, a condition not likely to be encountered in an actual fumigation (but provided for reference). The results indicate the following:

1. Table 11 shows that a greater than 4 $\log_{10}$ reduction was achieved at the 9000 ppmv-hr for *S. chartarum*, *A. versicolor*, and *A. alternata* on latex-painted wallboard, unpainted pine wood, carpet and glass. Likewise, a greater than 4 $\log_{10}$ reduction was achieved at the 9000 ppmv-hr for *C. globosum* and *A. fumigatus* on unpainted pine wood and carpet. A minimum 4 $\log_{10}$ (99.998% CFU count) reduction was achieved at the 9000 ppmv-hr for *C. globosum* and *A. fumigatus* on latex-painted wallboard and glass. A greater than 4 $\log_{10}$ reduction was achieved for *Stachybotrys* on all materials and all CTs from 3000 to 9000 ppmv-hr.

2. In most cases, there did not appear to be a significant difference between the efficacies of the two approaches to achieving the CT of 9000 ppmv-hr for most organisms. The possible exception is the *Alternaria* where the 3000 ppm for 3 hours appeared to be as much as one $\log_{10}$ more effective.

3. The fumigation appears to be somewhat more effective for a given organism on the nonvegetative coupons as compared to the same organism on a vegetative coupon. However, this determination may be an artifact of the higher CFU on the nonvegetative coupons for some organisms.

4. In virtually all cases where the coupons were treated at the target CT of 9000 ppmv-hr, no viable mold cells were detected, so the value was reported as ≤ the detection limit of the method, which ranged from $\log_{10}$ 0.88 to 2.00. This value represents a maximum number of remaining viable mold cells between 8 and 100.

5. If good mold remediation practices have been followed in the field, the source of water will have been removed, and the RH will be controlled. Under these circumstances, regrowth of the residual mold spores would not be expected. In some cases, although a ≥4 $\log_{10}$ reduction in CFU was attained, there were still viable spores on the coupons (data not shown). These spores may have the ability to germinate should the environmental conditions become or remain favorable. The germination potential of the spores was not evaluated.

6. The conclusion is therefore that ClO_2 fumigation at a CT of 9000 ppmv-hr may be an effective technique for reducing commonly occurring mold on typical building materials based upon laboratory testing. In many cases, the target reduction was achieved at lower CTs.

Allergen reduction: The hypothesis of the experiment was that chlorine dioxide fumigation had the potential to achieve significant reductions in the allergens and mycotoxins associated with all species. The reduction as a function of CT was evaluated on two types of samples: coupons of all four materials inoculated with pure *Alternaria* allergen Alt a 1 and coupons of the three non-vegetative materials inoculated with spores of *Alternaria*. The coupons were fumigated at CT values between 3000 and 9000 ppmv-hr. The results indicate the following:

1. For the pure allergen Alt a1, the concentration was reduced between 99.2 and 100 % at CT values of 4500 or greater on pine wood, carpet and glass coupons.

2. For the pure allergen Alt a1 on wallboard, the reduction was between 90.5 and 98.8% for CT values between 3000 and 9000 ppmv-hrs.

3. The allergens from the *Alternaria* CFU coupons were reduced between 98.6 to 100% on the three materials for CT values of 4500 and 9000 ppm-hrs.

4. There is no significant difference between the results for treatment at 750 ppm compared to the 3000 ppm treatment on the *Alternaria* CFU coupons.

5. The allergen Asp f 1 from the *A. fumigatus* latex-painted wallboard coupons showed a % inactivation ≥ 95 % and on unpainted pine wood showed a % inactivation ≥ 85.55 at a CT of 9000 ppmv-hr. These results suggest that the efficacy of allergen inactivation is material-dependent. The other two materials, wallpapered latex-painted wallboard and ceiling tiles, were tested only at CTs of 1125 and 2250, and the Asp f1 inactivation was very low.

6. In conclusion, allergen inactivation is dependent on the building material and the type of allergen.

Mycotoxin reduction: The coupons were fumigated at CT values between 3000 and 9000 ppmv-hr. The results indicate the following

1. The mycotoxin was reduced by 91.5 to 96.3 % for wallboard, pine and glass for CT values of 4500 ppm-hr and above.

2. The carpet appears to be the most difficult to fumigate with 92.3% reduction achieved only with the treatment at 3000 ppm for 3 hours.

3. If similar results were obtained on mycotoxins derived from vegetative mold species, high reductions of spore loadings by fumigation may provide effective remediation of mycotoxins and significantly reduce the likelihood of regeneration.

Overall conclusion: In any mold remediation, the EPA guidelines call for repairing the integrity of the structure and/or removing all other sources of water intrusion, as well as controlling RH. This research has demonstrated the potential to achieve large percentage reductions in viable spore counts on all materials as well as significant percentage reductions of the concentration of allergens and mycotoxins. This laboratory research showed that the results of allergen and mycotoxin inactivation are very promising. The building materials and most of the contents can be fumigated in place and effectively reused after the fumigation.

5. QUALITY ASSURANCE AND QUALITY CONTROL

The objective of this study was to determine the efficacy of chlorine dioxide fumigation for mold remediation. The Data Quality Objectives (DQOs) define the critical measurements (CMs) needed to address the stated objectives and specify tolerable levels of potential error associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- Real-time fumigant concentrations;
- Temperature;
- Relative humidity;
- Fumigation time sequence; and
- Mold, allergen, or mycotoxin counts.

Data Quality

The Quality Assurance Project Plan (QAPP) in place for this testing was followed with few deviations. Deviations included:

- In some of the runs, mycotoxin data were not reported because certain compounds in the building materials interfered with the mycotoxin assay. Water-soluble components in the materials may have inactivated the reagents. For this same reason, the polymerase chain reaction (PCR) method could not be used for analysis.

- Certain coupon materials, like glass, would not sustain mold growth, and in these cases the spores were inoculated directly onto the materials.

Data Quality Indicator Goals for Critical Measurements

The Data Quality Indicators (DQIs) listed in Table 12 are specific criteria used to quantify how well the collected data meet the DQOs for the fumigations.

Table 12. DQIs for Critical Measurements

Measurement Parameter	Analysis Method	Accuracy	Detection Limit	Completeness[1] %
Real-time ClO_2 concentration inside the test chamber	ClorDiSys Environmental Monitoring System (EMS) monitor (0.1 – 30 mg/L)	15% of modified SM-4500-E	0.1 mg/L 36 ppm	100
Extracted ClO_2, high concentration	Modified SM 4500-ClO_2 E	10% of standard	0.1 mg/L (solution)	90

Relative humidity	RH probes (0-100 %)	± 5 % of 83% standard salt solution	1%	95
Differential time	Computer clock	1 % of reading	0.5 sec	95
Temperature (T) inside the isolation chamber	Thermocouple	± 2 °C	NA	90

[1] Completeness goals of 100% are used for those parameters that are performed manually and infrequently. A completeness goal of 95% is used for those data streams that are automatically logged.

The measurements listed in Table 12 are specific criteria used to quantify how well the collected data met the DQOs. The accuracy of the real-time ClO_2 monitor was assessed with respect to the Modified SM 4500-ClO_2 E Method. Corrections to the real time concentration set-point were made so that the target concentration was attained according to the titration measurement. The accuracy of the extractive titration was assessed with respect to a standard solution.

The DQIs for the biological assays are found in Table 13.

Table 13. DQIs for the Assay Critical Measurements

Measurement Parameter	Frequency	Acceptable Criteria	Completeness %
Minimum counts from coupons	Each extraction	Minimum of 1 CFU/plate. If there are 0 CFU, 0.5 CFU will be used in the spreadsheet as the minimum detected.	95
Maximum counts from coupons	Each extraction	Maximum of 500 CFU/plate	95
Duplicate CFU	Each test, statistical check of data quality	Coefficient of variance ≤ 0.25	100

The DQIs for the allergen analysis are found in Table 14.

Table 14. DQIs for the Allergen Analysis Critical Measurements

Measurement Parameter	Frequency	Acceptable Criteria	Completeness %
Minimum acceptable allergen	Each ELISA analysis	Optical density at 405 nm must be equal to or greater than 15 ng and have a detection value within	95

		standard curve	
Maximum acceptable allergen	Each ELISA analysis	Optical density at 405 nm must be equal to or less 100 ng and have a detection value within standard curve	95
Duplicate samples	Each test, statistical check of data quality	Coefficient of variance ≤ 0.25	100

The DQIs for the mycotoxin analysis are found in Table 15.

Table 15. DQIs for the Mycotoxin Analysis Measurements

Measurement Parameter	Frequency	Acceptable Criteria	Completeness %
Minimum acceptable mycotoxin	Each ELISA analysis	Optical density detection value at 450 nm greater than or equal to 10 ng per reaction.	95
Maximum acceptable mycotoxin	Each extraction	Optical density detection value at 450 nm less than or equal to 100 ng per reaction	95
Duplicate Samples	Each test, statistical check of data quality	Coefficient of variance ≤ 0.25	100

The accuracy of the differential time was not assessed but error is expected to be negligible through the use of a computer clock. The accuracy of the thermocouple was determined through the measurement of the uncertainty following calibration.

All data from the ClO_2 fumigations satisfied the precision requirements.

Equipment Calibration

All equipment (e.g., pipets, incubators, biological safety cabinets) used at the time of evaluation was verified as being certified, calibrated, or validated. All equipment (e.g., pipets, thermocouples, RH probe, clocks) at US EPA was certified as being calibrated or having the calibration validated by EPA's on-site (RTP, NC) Metrology Laboratory at the time of use. Any

deficiencies were noted in the laboratory notebooks. Instruments were adjusted to meet calibration tolerances and recalibrated. If tolerances were not met after recalibration, additional corrective action was taken, possibly including, recalibration and/or replacement of the equipment. The MSM 4500-E was used to confirm the real-time EMS ClO_2 measurements.

Audits

This project was assigned QA Category III and did not require technical systems or performance evaluation audits.

6. REFERENCES

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RTI International Operating Procedures

MMBD SOP #001 Standard Operating Procedure for the Preparation of Media – Dehydrated.

MMBD SOP #002 Standard Operating Procedure for the Preparation of Sterile Water

MMBD SOP #003 Standard Operating Procedure for the Preparation of Sterile Buffer.

MMBD SOP #005 Standard Operating Procedure for the Characterization of Relative Humidity Chambers.

MMBD SOP #009 Standard Operating Procedure for Quantitative Evaluation of Microorganisms.

MMBD SOP #012 Standard Operating Procedure for Quantitation of Viable Microorganisms in Suspension.

MMBD SOP #017 Standard Operating Procedure for Decontamination of Humidity Chambers.

MMBD SOP #058 Standard Operating Procedure for Direct Inoculation of Materials with a Spore Suspension

All SOPs are maintained on file at RTI International and access to these files is permitted on-site at RTI International.

Appendix A: Tables of Results for Allergens and Mycotoxins in Alternate Format

Table A 1. Summary of Results for Pure Antigen rAlt a1 on All Materials (nonvegetative coupons)

ClO_2 concentration (ppmv)	Exposure time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (ng log_{10}) Mean ± St. Dev.	Exposed Coupons (ng log_{10}) Mean ± St. Dev.	Log Change (ng log_{10}) Mean ± St. Dev.
Latex-painted Gypsum Wallboard					
3000	1	3000	2.96 ± 0.04	0.66 ± 0.61	- 2.30 ± 0.43
750	6	4500	2.92 ± 0.01	1.85 ± 0.02	- 1.07 ± 0.02
750	9	6750	2.92 ± 0.01	1.90 ± 0.06	- 1.02 ± 0.04
750	12	9000	2.92 ± 0.01	1.91 ± 0.05	- 1.01 ± 0.04
3000	3	9000	2.96 ± 0.04	1.03 ± 0.48	- 1.93 ± 0.34
Unpainted Pine Wood					
3000	1	3000	2.78 ± 0.08	1.87 ± 0.34	- 0.91 ± 0.24
750	6	4500	2.85 ± 0.14	≤ 0.00 ± 0.00	≥ - 2.85 ± 0.10
750	9	6750	2.85 ± 0.14	≤ 0.00 ± 0.00	≥ - 2.85 ± 0.10
750	12	9000	2.85 ± 0.14	≤ 0.00 ± 0.00	≥ - 2.85 ± 0.10
3000	3	9000	2.78 ± 0.08	0.66 ± 0.51	- 2.12 ± 0.37
Carpet					
750	6	4500	2.92 ± 0.01	0.31 ± 0.56	- 2.61 ± 0.39
750	9	6750	2.92 ± 0.01	0.12 ± 0.76	- 2.80 ± 0.54
750	12	9000	2.92 ± 0.01	0.15 ± 0.24	- 2.77 ± 0.17
3000	3	9000	2.97 ± 0.08	0.49 ± 0.46	- 2.48 ± 0.33
Glass					
750	6	4500	2.89 ± 0.05	≤ 0.00 ± 0.00	≥ - 2.89 ± 0.04
750	9	6750	2.89 ± 0.05	≤ 0.00 ± 0.00	≥ - 2.89 ± 0.04
750	12	9000	2.89 ± 0.05	≤ 0.00 ± 0.00	≥ - 2.89 ± 0.04
3000	3	9000	2.76 ± 0.09	0.31 ± 0.54	- 2.45 ± 0.38

Table A 2. Summary of Results for Pure Aflatoxin on All Materials (nonvegetative coupons)

ClO_2 concentration (ppmv)	Exposure time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (ng log_{10}) Mean ± St. Dev.	Exposed Coupons (ng log_{10}) Mean ± St. Dev.	Log Change (ng log_{10}) Mean ± St. Dev.
Latex-painted Gypsum Wallboard					
750	1.5	1125	1.95 ± 0.03	1.39 ± 0.20	- 0.56 ± 0.12
750	3	2250	1.95 ± 0.03	1.07 ± 0.06	- 0.88 ± 0.05
3000	1	3000	1.82 ± 0.15	1.17 ± 0.12	- 0.64 ± 0.14
750	6	4500	2.02 ± 0.04	0.84 ± 0.13	- 1.18 ± 0.10
750	9	6750	2.02 ± 0.04	0.65 ± 0.08	- 1.37 ± 0.06
750	12	9000	2.02 ± 0.04	≤ 0.60 ± 0.00	≥ - 1.42 ± 0.03
3000	3	9000	1.82 ± 0.15	0.63 ± 0.04	- 1.19 ± 0.11
Wallpapered Latex-painted wallboard					
750	1.5	1125	1.85 ± 0.32	1.51 ± 0.40	- 0.34 ± 0.36
750	3	2250	1.85 ± 0.32	1.38 ± 0.46	- 0.47 ± 0.39
Ceiling Tile					
750	1.5	1125	2.49 ± 0.14	1.04 ± 0.02	- 1.45 ± 0.08
750	3	2250	2.49 ± 0.14	1.02 ± 0.03	- 1.47 ± 0.09
Unpainted Pine Wood					
750	1.5	1125	1.67 ± 0.09	≤ 0.90 ± 0.00	≥ - 0.77 ± 0.05
750	3	2250	1.67 ± 0.09	0.91 ± 0.01	-0.76 ± 0.05
3000	1	3000	1.83 ± 0.15	≤ 0.60 ± 0.00	≥ - 1.23 ± 0.11
750	6	4500	1.84 ± 0.17	0.66 ± 0.04	- 1.17 ± 0.12
750	9	6750	1.84 ± 0.17	0.65 ± 0.02	- 1.19 ± 0.12
750	12	9000	1.84 ± 0.17	0.63 ± 0.03	- 1.20 ± 0.12
3000	3	9000	1.83 ± 0.15	≤ 0.60 ± 0.00	≥ - 1.23 ± 0.11
Carpet					
750	6	4500	1.71 ± 0.08	1.42 ± 0.10	- 0.29 ± 0.09
750	9	6750	1.71 ± 0.08	0.97 ± 0.16	- 0.74 ± 0.13
750	12	9000	1.71 ± 0.08	0.82 ± 0.19	- 0.89 ± 0.15
3000	3	9000	2.00 ± 0.06	0.89 ± 0.09	- 1.10 ± 0.08
Glass					
750	6	4500	1.68 ± 0.06	≤ 0.60 ± 0.00	≥ - 1.08 ± 0.04
750	9	6750	1.68 ± 0.06	≤ 0.60 ± 0.00	≥ - 1.08 ± 0.04
750	12	9000	1.68 ± 0.06	≤ 0.60 ± 0.00	≥ - 1.08 ± 0.04
3000	3	9000	2.19 ± 0.19	0.74 ± 0.29	- 1.45 ± 0.24

Table A 3. Summary of Results for Asp f 1 Allergen from *Aspergillus fumigatus* CFU Inoculated Positive Controls and Exposed Coupons in All Materials (vegetative coupons)

ClO_2 concentration (ppmv)	Exposure time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons* (ng log_{10}) Mean ± St. Dev.	Exposed Coupons* (ng log_{10}) Mean ± St. Dev.	Log Change (ng log_{10}) Mean ± St. Dev.
Latex-painted Gypsum Wallboard					
750	1.5	1125	-0.58 ± 0.14	-0.52 ± 0.31	0.05 ± 0.24
750	3	2250	-0.58 ± 0.14	≤ - 0.40 ± 0.00	0.18 ± 0.10
3000	3	9000	0.92 ± 0.51	≤ - 0.40 ± 0.00	≥ - 1.31 ± 0.36
Wallpapered Latex-painted wallboard					
750	1.5	1125	-0.72 ± 0.24	-0.65 ± 0.32	0.06 ± 0.28
750	3	2250	-0.72 ± 0.24	-0.52 ± 0.30	0.20 ± 0.27
Ceiling Tile					
750	1.5	1125	-0.88 ± 0.14	-0.92 ± 0.26	- 0.04 ± 0.21
750	3	2250	-0.88 ± 0.14	-0.50 ± 0.26	0.38 ± 0.21
Unpainted Pine Wood					
750	1.5	1125	-0.76 ± 0.40	-0.75 ± 0.39	0.01 ± 0.39
750	3	2250	-0.76 ± 0.40	-0.75 ± 0.39	0.01 ± 0.39
3000	3	9000	0.44 ± 0.49	≤ - 0.40 ± 0.00	≥ - 0.84 ± 0.35

Asp f 1 allergen data are not shown for carpet and glass because the allergen is a hyphal allergen.

* Note that because these data are log_{10}, a negative value is a number less than 1 (e.g., 0.8 ng = -0.1 ng log_{10})

Table A 4. Summary of Results for Alt a 1 Allergen from *Alternaria alternata* CFU Inoculated Positive Controls and Exposed Coupons on All Materials (nonvegetative coupons)

ClO_2 concentration (ppmv)	Exposure time (hr)	Anticipated Total Exposure - CT (ppmv-hr)	Control Coupons (ng log_{10}) Mean ± St. Dev.	Exposed Coupons (ng log_{10}) Mean ± St. Dev.	Log Change (ng log_{10}) Mean ± St. Dev.
Unpainted Pine Wood					
750	6	4500	2.20 ± 0.33	0.55 ± 0.29	-1.65 ± 0.31
750	12	9000	2.20 ± 0.33	0.35 ± 0.54	-1.86 ± 0.45
3000	3	9000	2.25 ± 0.07	0.05 ± 0.11	-2.20 ± 0.09
Carpet					
750	6	4500	2.95 ± 0.06	0.56 ± 0.31	-2.39 ± 0.32
750	12	9000	2.95 ± 0.06	0.19 ± 0.42	-2.77 ± 0.38
3000	3	9000	1.87 ± 0.37	0.06 ± 0.14	-1.82 ± 0.11
Glass					
750	6	4500	2.56 ± 0.25	0.55 ± 0.35	-2.00 ± 0.34
750	12	9000	2.56 ± 0.25	0.57 ± 0.33	-1.98 ± 0.33
3000	3	9000	1.95 ± 0.16	≤ 0.00 ± 0.00	≥ -1.95 ± 0.05

Alt a 1 allergen data are not shown for latex-painted gypsum wallboard due to material assay interference or insufficient spore production on the growing sample.

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