

Laboratory Research on the Efficacy of Chlorine Dioxide for the Remediation of Mold-Contaminated Buildings

Doris Betancourt, Ph.D.
Shannon Serre, Ph.D.
Timothy Dean, Ph.D.
G Blair Martin (retired)
US EPA/ORD, RTP, NC

John Y Mason
Sabre Technical Services

Acknowledgements

- Dave Mickunas, US EPA - presenter
- Shawn Ryan, US EPA – Director DCMD
- Karin Foarde, RTII – biological analysis

CRADA Overview

- Chlorine Dioxide fumigation has been successful in remediating a number of biological contaminated buildings
 - 4 large buildings contaminated with *B. anthracis* in 2001
 - Numerous mold contaminated buildings following storm damage
 - Other bacteria and viruses in commercial buildings
 - In a variety of small scale R & D studies
- The mold remediations were performed under FIFRA 24(C) state registrations
- Full FIFRA registration requires supporting R & D data
- The USEPA has facilities to perform fumigation and biological analysis
- Sabre Technical Services entered into a Cooperative Research and Development Agreement (CRADA) with EPA in RTP, NC for a systematic study of ClO₂ for mold remediation

Presentation Overview

- Objectives
- Materials and Methods
- Results
- Discussion

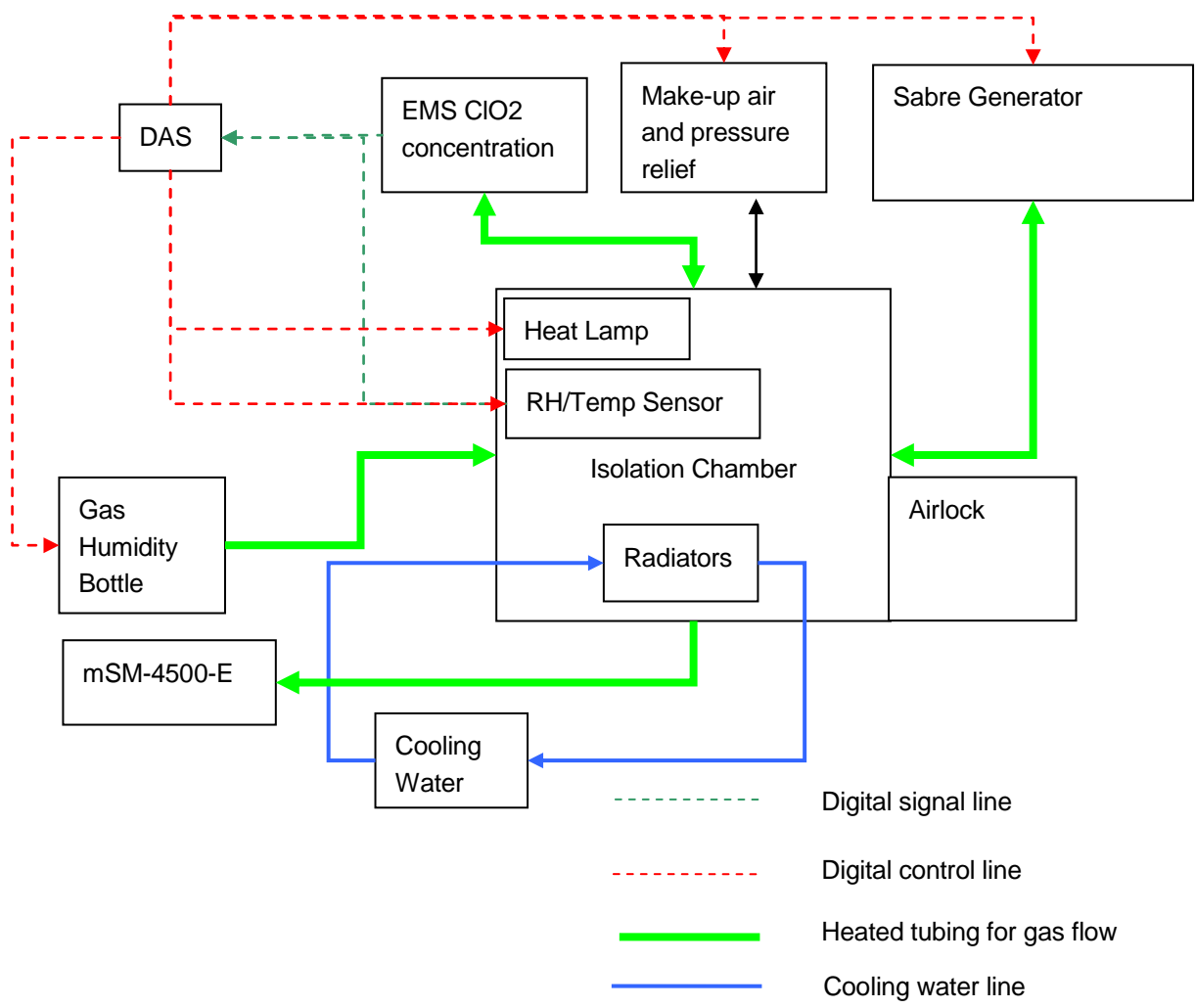
Objectives

- To determine the ability of ClO_2 to inactivate/denature culturable fungi, mycotoxins and allergens on building materials fumigated by ClO_2 vapor at low and high concentrations over several time periods

Materials and Methods

- ClO₂ gas generated using a Sabre Technical Services patented generator (Albany, NY). Two exposure concentrations tested – 750ppmv ClO₂ and 3000ppmv ClO₂
- Up to five exposure times resulting in total ClO₂ exposures (CT) of 2250, 3000, 4500, 6750, and 9000 ppmv-hr. (CT = ClO₂ conc x time)

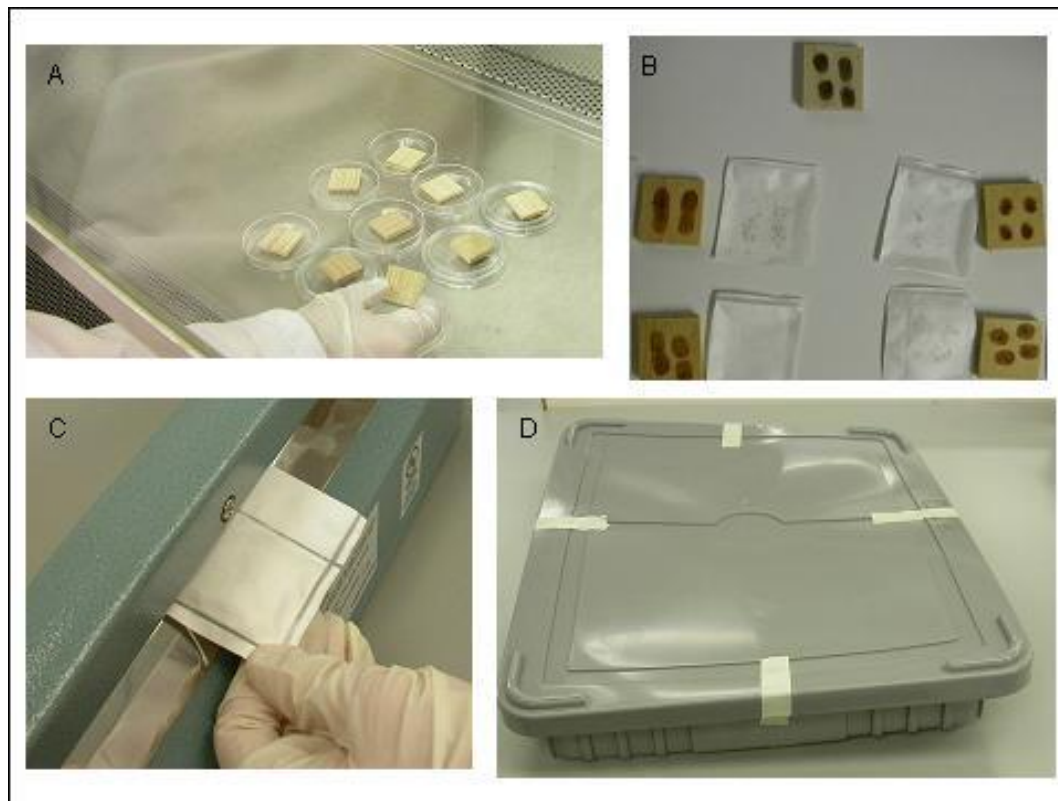
EXPERIMENTAL FUMIGATION SYSTEM



Materials and Methods

- Test materials: wood, latex painted gypsum wallboard, carpet and glass. Coupons: 1" by 1" squares. Two types of coupons prepared: vegetative and non-vegetative coupons
- Mold spores used: *Aspergillus versicolor*, *Aspergillus fumigatus*; *Stachybotrys chartarum*; *Chaetomium globosum*, and *Alternaria alternata*. Spores' conc./coupon: $10^6 - 10^7$
- Allergen: *Alternaria* antigen rAlt a1 from pure Ag and from *A.alternata* CFU inoculated coupons and Asp f1 from *A.fumigatus* from CFU inoculated coupons
- Mycotoxin: Aflatoxin B1

Coupon preparation process



Static chambers used for preparation of vegetative coupons



Materials and Methods

Analyses performed:

- **Culturability assay:** inactivation of culturable fungi:

$$\text{Log}_{10}\text{CFU}_{\text{change}} = \text{Log}_{10}\text{CFU}_{\text{C}} - \text{Log}_{10}\text{CFU}_{\text{E}}$$

Success Criteria: 4 log reduction (99.99% CFU reduction)

Materials and Methods

- **Mycotoxin analysis:** Aflatoxin on coupons - measured by ELISA

Allergen analysis (ELISA): inactivation of

- Purified *Alternaria* antigen rAlt a1 inoculated on coupons
- rAlt a1 from *A.alternata* CFU and Asp f1 from *A.fumigatus* CFU on inoculated coupons
- To quantify the efficacy of ClO₂ to inactivate **mycotoxins**, and **allergens**: mycotoxin and allergen were calculated

$$\text{Log}_{10} \text{ ng change} = \text{Log}_{10} \text{ ng}_C - \text{Log}_{10} \text{ ng}_E$$

Success Criteria: % reduction of mass

Test Matrix for ClO₂ Fumigation

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Test ID	Materials	Contamination	Treatment	Time Points (hr)	Analysis	Total Coupons
Run 1	G, L, W, C	Stachybotrys	none (pos.control)	0	V(6), M(6), A(6)	72
	G, L, W, C	none (blank)	ClO ₂ (neg. cont.)	6, 12	V(1), M(1), A(1)	24
	G, L, W, C	Stachybotrys	750 ppmv ClO ₂	6, 12	V(6), M(6), A(6)	144
Run 2	W	Chaetomium	none (pos.control)	0	V(6)	6
	W	none (blank)	7 ClO ₂ (neg. cont.)	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. cont.)	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. cont.)	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	allergen (C1)	none (pos.control)	0	A(6)	24
	G, L, W, C	none (blank)	ClO ₂ (neg. cont.)	6, 9, 12	A(6)	12
	G, L, W, C	allergen (C1)	750 ppmv ClO ₂	6, 9, 12	A(6)	72
Run 6	G, L, W, C	Aspergillus	none (pos.control)	0	V(6), M(6), A(6)	72
	G, L, W, C	none (blank)	ClO ₂ (neg. cont.)	6, 12	V(1), M(1), A(1)	24
	G, L, W, C	Aspergillus	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. cont.)	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	G, L, W, C	1 (L&W), 3 (all)	M(1)	6
	G, L, W, C	mycotoxin (C2)	G, L, W, C	1 (L&W), 3 (all)	M(6)	36
Run 9	G, L, W, C	allergen (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	allergen (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. cont.)	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	none (pos.control)	0	V(6), M(6), A(6)	72
	G, L, W, C	none (blank)	ClO ₂ (neg. cont.)	3	V(1), M(1), A(1)	12
	G, L, W, C	Aspergillus	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. cont.)	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. cont.)	3	V(1), A(1)	8
	G, L, W, C	Alternaria	3000 ppmv ClO ₂	3	V(6), A(6)	48

Building a
scientific
foundation
for sound
environmental
decisions

Test Matrix for ClO₂ Fumigation

Test ID	Materials	Contamination	Treatment	Time Points (hr)	Analysis	Total Coupons
Run 1	G, L, W, C	Stachybotrys	none (pos.control)	0	V(6), M(6), A(6)	72
	G, L, W, C	none (blank)	ClO ₂ (neg. cont.)	6, 12	V(1), M(1), A(1)	24
	G, L, W, C	Stachybotrys	750 ppmv ClO ₂	6, 12	V(6), M(6), A(6)	144
Run 2	W	Chaetomium	none (pos.control)	0	V(6)	6
	W	none (blank)	ClO ₂ (neg. cont.)	3, 6, 9, 12	V(1)	4
	W	Chaetomium	750 ppmv ClO ₂	3, 6, 9, 12	V(6)	24

V = viability ; M = mycotoxin; A= allergen

Concentration (C)	Time (T)	Total ppmv*hr (C*T)
ppmv	hr	ppmv-hr
750	3	2250
750	6	4500
750	9	6750
750	12	9000
3000	3	9000

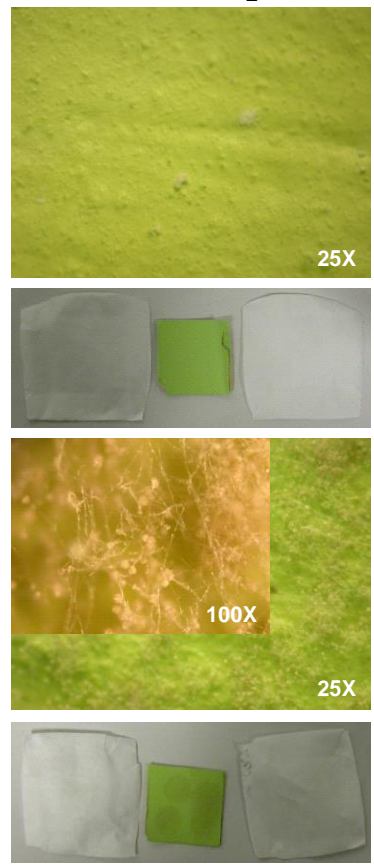
RESULTS

Wallboard Results

Table 1. Summary of Results for Latex Painted Wallboard Vegetative Coupons						
CT ppmv- hrs	Control Coupons		Treated Coupons		LOG Reduction	CFU Reduction %
	LOG10	CFU	LOG10	CFU		
Stachybotrys chartarum						
3000	6.75	5.6x10 ⁶	≤2.0	≤100	4.75	≥99.998
4500	6.78	6.0x10 ⁶	≤2.0	≤100	4.78	≥99.998
9000	6.78	6.0x10 ⁶	≤2.0	≤100	4.78	≥99.998
9000	6.75	5.6x10 ⁶	≤2.0	≤100	4.75	≥99.998
Chaetomium globosum						
2250	5.67	5.7x10 ⁵	3.42	2600	2.23	99.5
3000	5.90	7.9x10 ⁵	2.46	288	3.44	99.96
9000	5.67	5.7x10 ⁵	1.73	54	3.94	99.99
9000	5.90	7.9x10 ⁵	1.35	22	4.55	99.997
Aspergillus versicolor						
4500	6.67	4.7x10 ⁶	1.93	85	4.74	99.998
9000	6.67	4.7x10 ⁶	≤1.88	76	4.79	≥99.998
Aspergillus fumigatus						
9000	7.46	2.9x10 ⁷	2.15	141	5.31	99.999
Alternaria alternata						
4500	3.90	7.9x10 ³	≤0.88	8	3.02	≥99.9
9000	3.90	7.9x10 ³	≤0.88	8	3.02	≥99.9
9000	4.23	1.7x10 ⁴	≤0.88	8	3.35	≥99.95

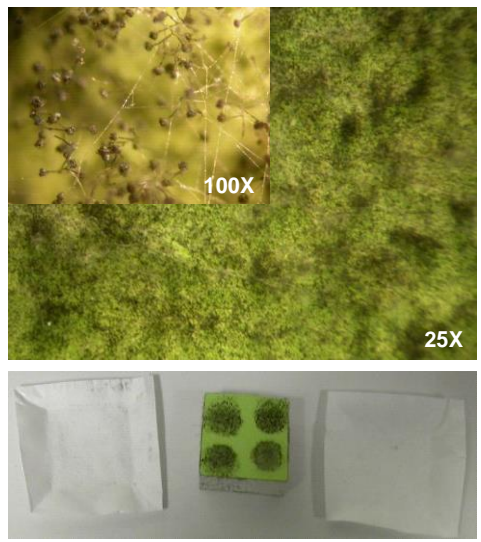
Stachybotrys chartarum on latex painted gypsum

6 hr ClO₂

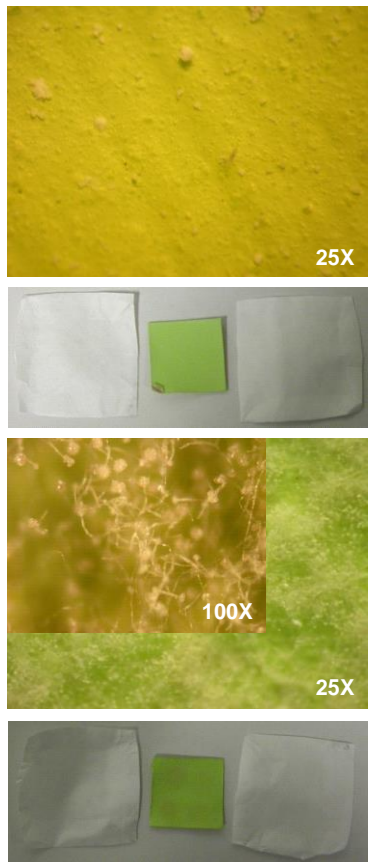


Stachybotrys chartarum on latex painted gypsum

Positive Control No ClO₂



12 hr ClO₂

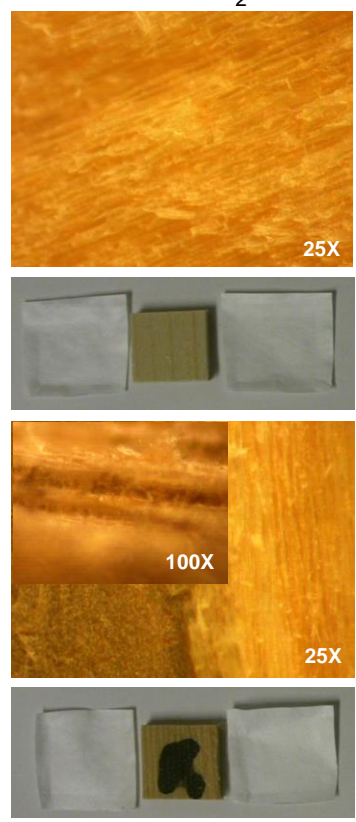


Unpainted Pine Results

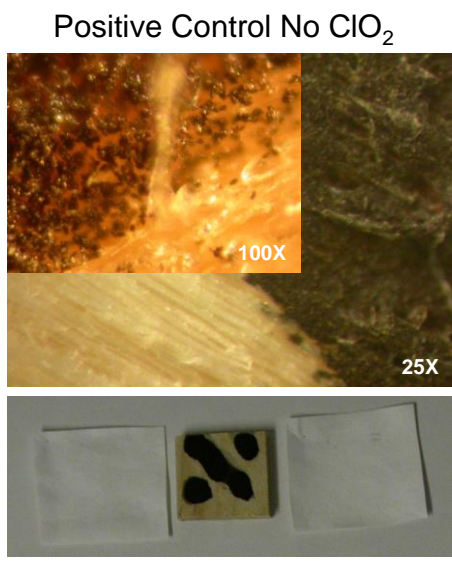
Table 2. Summary of Results for mixed growth unpainted pine wood Coupons						
CT ppmv- hrs	Control Coupons		Treated Coupons		LOG Reduction	CFU Reduction %
	LOG10	CFU	LOG10	CFU		
Nonegetative <i>Stachybotrys chartarum</i>						
3000	7.63	4.9x10 ⁷	≤2.0	≤100	≥5.63	≥99.9998
4500	7.24	1.7x10 ⁷	≤2.0	≤100	≥5.24	≥99.999
9000	7.24	1.7x10 ⁷	≤2.0	≤100	≥5.24	≥99.999
9000	7.63	4.9x10 ⁷	≤2.0	≤100	≥5.63	≥99.9998
Vegetative <i>Chaetomium globosum</i>						
2250	5.39	2.5x10 ⁵	0.93	8	4.46	99.997
3000	5.20	1.6x10 ⁵	0.98	9	4.22	99.99
4500	5.39	2.5x10 ⁵	1.03	11	4.36	99.996
6750	5.39	2.5x10 ⁵	≤0.88	≤8	≥4.51	≥99.997
9000	5.39	2.5x10 ⁵	≤0.88	≤8	≥4.51	>99.997
9000	5.20	1.69x10 ⁵	≤0.88	≤8	≥4.55	≥99.99
Vegetative <i>Aspergillus versicolor</i>						
4500	6.23	1.7x10 ⁶	1.93	85	4.30	99.995
9000	6.23	1.7x10 ⁶	≤1.88	≤80	≥4.35	≥99.995
Vegetative <i>Aspergillus fumigatus</i>						
9000	6.56	3.6x10 ⁶	≤2.0	≤100	≥4.56	≥99.997
Nonvegetative <i>Alternaria alternata</i>						
4500	6.29	2.0x10 ⁶	≤1.88	≤80	≥4.41	≥99.996
9000	6.29	2.0x10 ⁶	≤1.88	≤80	≥4.41	≥99.996
9000	7.01	1.0x10 ⁷	≤1.88	≤80	≥4.89	≥99.999

Stachybotrys chartarum on wood

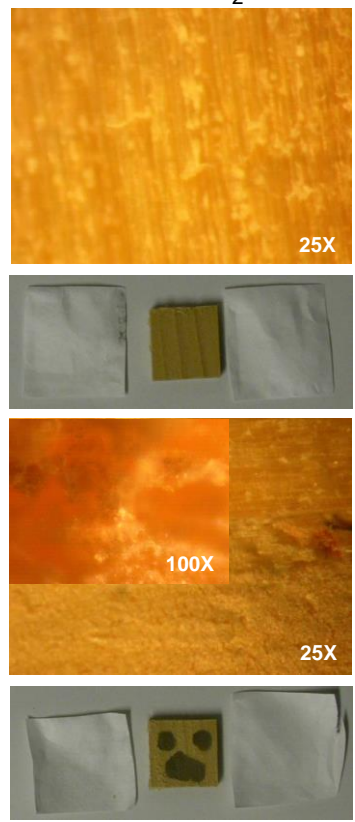
6 hr ClO₂



Stachybotrys chartarum on wood



12 hr ClO₂

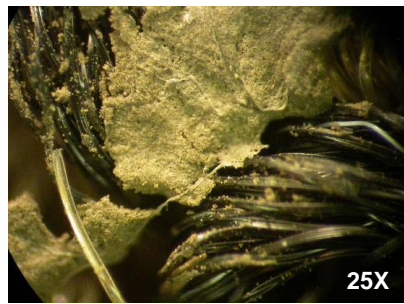


Carpet Results

Table 3. Summary of Results for Carpet Non-Vegetative coupons							
CT ppmv- hrs	Control Coupons		Treated Coupons		LOG Reduction	CFU Reduction %	
	LOG10	CFU	LOG10	CFU			
Stachybotrys chartarum							
4500	7.15	1.1x10 ⁷	2.32	210	4.83	99.999	
9000	7.15	1.4x10 ⁷	2.04	110	5.11	99.9995	
9000	7.46	2.9x10 ⁷	≤2.00	≤100	≥5.46	≥99.9996	
Chaetomium globosum							
2250	7.19	1.5x10 ⁷	3.70	5000	3.49	99.97	
9000	7.19	1.5x10 ⁷	2.18	150	5.01	99.999	
9000	7.64	4.4x10 ⁷	2.62	410	5.02	99.999	
Aspergillus versicolor							
4500	6.94	8.74x10 ⁶	≤1.88	≤80	≥5.06	≥99.999	
9000	6.94	8.74x10 ⁶	≤1.88	≤80	≥5.06	≥99.999	
Aspergillus fumigatus							
9000	7.16	1.4x10 ⁷	≤1.88	≤80	≥5.28	≥99.999	
Alternaria alternata							
4500	5.93	8.5x10 ⁵	≤1.88	≤80	≥4.05	≥99.99	
9000	5.93	8.5x10 ⁵	≤1.88	≤80	≥4.05	≥99.99	
9000	6.91	8.1x10 ⁶	≤1.88	≤80	≥5.03	≥99.999	

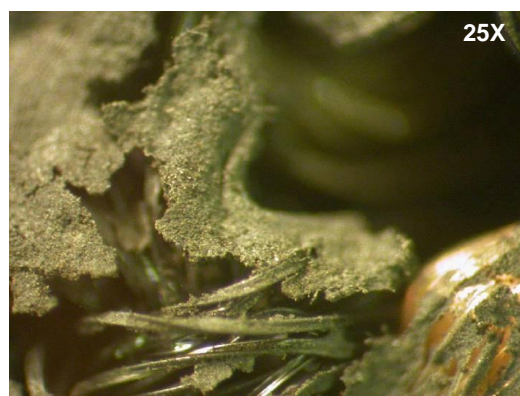
Stachybotrys chartarum on carpet

6 hr ClO₂

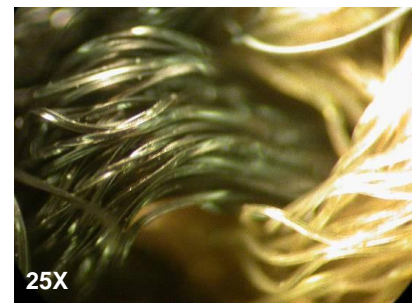


Stachybotrys chartarum on carpet

Positive Control No ClO₂



12 hr ClO₂



Pure Allergen Results

Table 4. Summary for Pure Alternaria Alt a 1 Allergen on Materials

CTC PPM Hrs	Control Coupons		Treated Coupons		Log10 Reduction	Mass Reduction %
	LOG10	ng	LOG10	ng		
Latex painted wallboard						
3000	2.96	910	0.66	4.6	2.30	99.5
4500	2.92	830	1.85	71	1.07	91.4
6750	2.92	830	1.90	79	1.02	90.5
9000	2.92	830	1.91	81	1.01	90.2
9000	2.96	910	1.03	11	1.93	98.8
Unpainted Pine Wood						
3000	2.78	600	1.87	74	0.89	87.7
4500	2.85	710	≤0.00	0.0	≥2.85	100
6750	2.85	710	≤0.00	0.0	≥2.85	100
9000	2.85	710	≤0.00	0.0	≥2.85	100
9000	2.78	600	0.66	4.6	2.12	99.2
Carpet						
4500	2.92	830	0.31	2.0	2.61	99.8
6750	2.92	830	0.12	1.1	2.80	99.9
9000	2.92	830	0.15	1.1	2.77	99.9
9000	2.97	930	0.49	3.1	2.47	99.7
Glass						
4500	2.89	780	≤0.00	0.0	≥2.89	100
6750	2.89	780	≤0.00	0.0	≥2.89	100
9000	2.89	780	≤0.00	0.0	≥2.89	100
9000	2.76	580	0.31	2.0	≥2.45	>99.7

Alternaria Allergen Results

Table 5. Summary for Alt a 1 Allergen from Alternaria vegetative coupons							
CTC PPM Hrs	Control Coupons		Treated Coupons		Log10 Reduction	Mass Reduction %	
	LOG10	ng	LOG10	ng			
Unpainted Pine							
4500	2.20	160	0.55	3.5	1.65	97.8	
9000	2.20	160	0.35	2.2	1.85	98.6	
9000	2.25	180	0.09	1.1	2.16	99.4	
Carpet							
4500	2.95	890	0.56	3.6	2.39	99.6	
9000	2.95	890	0.19	1.5	2.76	99.8	
9000	1.87	74	0.05	1.1	1.82	98.5	
Glass							
4500	2.55	360	0.55	3.5	2.00	99.0	
9000	2.56	360	0.57	3.7	1.99	99.0	
9000	1.95	91	≤0.00	0.0	≥1.99	100	

Pure Mycotoxin Results

Table 6. Summary for Pure Aflatoxin on Materials							
CTC PPM Hrs	Control Coupons		Treated Coupons		Log10 Reduction	Mass Reduction %	
	LOG10	ng	LOG10	ng			
Latex Painted Wallboard							
3000	1.82	66	1.17	15	0.65	77	
4500	2.02	100	0.84	6.9	1.18	93.1	
6750	2.02	100	0.65	4.5	1.37	95.5	
9000	2.02	100	≤0.60	≤4.0	≥1.42	≥96.0	
9000	1.82	66	0.63	≤4.2	1.19	93.7	
Unpainted Pine							
3000	1.83	67	≤0.60	≤4.0	≥1.23	≥94.0	
4500	1.84	69	0.66	4.6	1.18	93.3	
6750	1.84	69	0.65	4.5	1.19	93.4	
9000	1.84	69	0.63	4.2	1.21	93.5	
9000	1.83	67	≤0.60	≤4.0	≥1.23	≥94.0	
Carpet							
4500	1.71	51	1.42	26.0	0.29	49.0	
6750	1.71	51	0.97	9.3	0.74	81.7	
9000	1.71	51	0.82	6.7	0.83	86.9	
9000	2.00	100	0.89	7.7	1.11	92.3	
Glass							
4500	1.68	47	≤0.60	≤4.0	≥1.26	≥91.5	
6750	1.68	47	≤0.60	≤4.0	≥1.26	≥91.5	
9000	1.68	47	≤0.60	≤4.0	≥1.26	≥91.5	
9000	2.19	150	0.74	5.5	1.45	96.3	

Conclusions - Molds

- Most treated coupons did not contain detectable CFU - reported as \leq the detection limit of the method, which ranged from $\leq \log 0.88$ to 2.00 (8 to 100 CFU)
- The target 4 log reduction was achieved at the 9000 ppmv-hrs for all organisms on all materials, except *Alternaria* on wallboard
- Only ≤ 4 log CFU of *Alternaria* were present on the wallboard positive control
- Greater than 4.75 log (99.999% CFU count) reduction was achieved for *Stachybotrys* on all materials and all CTs from 3000 to 9000 ppmv-hrs;

Conclusions – Allergens

- For the pure allergen on pine carpet and glass coupons, the concentration was reduced between 99.2 and 100 % at CT values of 4500 or greater. The residual concentrations ranged from 0.0 to 4.6 ng for these conditions
- For the pure allergen on wallboard, the reduction was between 90.5 and 98.8% for CT values between 3000 and 9000 ppmv-hrs.
- The allergens from the Alternaria CFU inoculated coupons were reduced between 97.8 and 100% on all three materials for CT values of 4500 and 9000 ppm-hrs

Cconclusions - Mycotoxins

- The mycotoxin was reduced by 91.5 to 96.3 % for wallboard, pine and glass for CT values of 4500 ppm-hrs and above.
- The carpet appears to be the most difficult to fumigate with the 92.3% reduction only achieved with the treatment at 3000 ppm for 3 hours

Overall Conclusions

- If good mold remediation practices have been followed, the source of water will have been removed and the relative humidity will be controlled. Under these circumstances, regrowth of the residual mold spores would not be expected.
- It does not appear likely with additional allergens would be generated
- The organic residue from the mold should be removed by vacuuming to remove potential irritants.
- High reductions of spore loadings by fumigation should provide effective remediation of any mycotoxins present and significantly reduce the likelihood of regeneration
- Therefore, chlorine dioxide fumigation at CT's of 9000 ppmv-hrs , or less, should be an effective technique for remediation of commonly occurring mold, allergens and mycotoxins on typical building materials.

Thank you

Questions?