Concentra
Environmental, Health, and Safety Services

May 8, 2008

Jon Lampros
President
Clean Air Systems
701 Rockland Rd.
Lake Bluff, IL 60044

Re: Assessment of Ozone Treatment on Microbial Populations

Dear Mr. Lampros,

The attached report is the result of a mold and bacterial assessment conducted at 750 4th Street, Franklin, Ohio on April 24 and 25, 2008.

You contacted Concentra Environmental, Health, and Safety Services (CEHS) with an interest in determining the effectiveness of ozone treatment on potential indoor fungal and bacterial contamination. The ozone system developed by Clean Air systems was used in this study. This system consists of a protocol in which ozone concentrations are produced using ultraviolet ozone generators for a period of time until a predetermined ozone level is reached in the area of interest.

Quantus conducted total airborne mold spore, airborne culturable fungi, and total surface bacterial sampling in an effort to determine the effect of ozone on bacterial and fungal populations present in the [redacted] High School men's locker room. Microbial sampling was conducted both before and after ozone treatment.

Measured total airborne fungal spores, culturable fungi, and total heterotrophic bacteria concentrations were consistently reduced after ozone treatment. Reduction of culturable fungi concentrations ranged from 95-100%, and total fungal spores were reduced by 38-97%. Furthermore, while reduction of bacterial concentrations varied with the sampled surface, concentrations were dramatically decreased in the shower area (99.5%).

In summary, results obtained during this survey indicate that the treatment was effective in reducing a variety of microbial contamination. Furthermore, the largest reduction in total airborne *Penicillium* spores in the shower area indicates that cellular structures were not only inactivated but physically destroyed. The efficacy of the treatment remains to be evaluated with better certainty throughout a more comprehensive assessment. This assessment would include determining variability of microbial concentrations with respect to ozone levels. A summary of *all* results can be found in Table 7 of this report.

At this time, no statements are being made as to whether fungal or bacteria sources do or do not exist in any un-sampled areas. Should you have any questions, please don't hesitate to call us (513) 871-5400.

Most Sincerely,

Javier Ortega, MS
Senior Environmental Toxicologist

Impact of Airborne Ozone on Indoor Fungal and Bacterial Populations

Survey Date: April 24 and 25, 2008

Survey Location:

[redacted]
[redacted] OH 45005

Site Survey and Reporting By:

Javier Ortega, MS
Senior Environmental Toxicologist

Project ID#: 3826

SURVEY LOCATION: [redacted] OH 45005

SURVEY DATE: April 24 & 25, 2008

CONTACT: Jon Lampros, President of Clean Air Systems

PURPOSE:

Concentra Environmental, Health, and Safety Services (CEHS) was contracted by Jon Lampros to assess the impact of elevated ozone concentration on indoor fungal and bacterial populations. High ozone concentrations were generated in the [redacted] High School men's locker room in an effort to reduce or eliminate viable fungi and bacteria that might be present as contaminants. Sampling data and conclusions are presented in the attached report.

BACKGROUND:

CEHS was informed by Mr. Lampros that his crew would perform ozone treatment intended to eliminate airborne and surface microorganisms that may pose health risks to [redacted] High School students. This school was selected because of recently reported cases of germ-acquired diseases. In order to assess the effectiveness of ozone treatment, CEHS measured 1) viable airborne fungal concentrations, 2) total airborne fungal concentrations, and 3) viable surface bacterial contamination before and after ozone treatment.

Paired air sample sets for the characterization of both diversity and concentration of total airborne mold spores and culturable fungi were collected from the shower and locker areas respectively. Surface composite samples were collected from the same areas for bacterial analysis. In order to compare microbial measurements, samples were collected before and after ozone treatment in the same locations, under identical conditions, i.e., sampling volume, surface, etc. High ozone concentrations were produced using ultraviolet ozone generators for several hours until a predetermined ozone level was reached. Ozone treatment was performed in accordance with protocol established by Clean Air Systems. After the treatment was complete, ozone generators were turned off and the area was ventilated by opening the access door and a ceiling latch to exhaust the remaining ozone from the locker room. CEHS was hired to perform a professional onsite mold and bacterial assessment.

FINDINGS AND OBSERVATIONS:

Airborne Mold

1. *Bioaerosols:* Before and after ozone treatment, a total of three air sample sets were collected for the determination and identification of airborne mold spores. Two paired sample sets were collected side by side to determine culturable fungi and total airborne spore concentrations. Samples were collected in 1) the shower area (figure 1 - 3) and 2) the

locker area (figure 4). An outdoor sample pair was collected to provide a baseline measurement against which to compare what was found in the indoor air.

Total airborne mold before treatment

In a building free of a mold reservoir, or contamination, the total indoor mold spore concentration of samples collected indoors will typically be equal to or less than outdoor concentration. Additionally, the types mold spores present indoors should be similar to those measured outdoors. In a contaminated property, however, the total mold spore concentration will typically be higher and/or the relative distribution of mold genera will be notably different than in the sample collected for comparison purposes.

While total airborne mold spore concentrations varied indoors (concentrations in the locker room were lower than outdoors; concentrations in the shower area were greater than outdoors), the relative distribution of spore types measured in all indoor samples was notably different than those measured outdoors. Difference among all fungal profiles was largely attributable to ***Aspergillus/Penicillium*** type concentrations. This mold group comprised only 3.9% of the outdoor air, but ranged from 87-98% of the sample composition indoors. Additionally, *Apergillus/Penicillium* type levels in the shower area and locker area were 103 and 17 times higher than that measured outdoors, respectively. These results indicated the presence of a mold source in the surveyed areas. See Table 1 for complete results.

Total airborne mold after treatment

Following ozone treatment, the concentration of *Aspergillus/Penicillium* type spores in the shower area was reduced by 97% with respect to the pre-treatment concentration, with a 38% reduction in the locker area. It should be noted that introduction of outdoor air to assist in exhausting ozone from the locker area likely resulted in the smaller spore reduction in the locker area. This area is close to a ceiling latch which was opened to the exterior immediately after the ozone treatment to assist in ventilating the facility. See Table 2 for complete results.

Culturable fungi before treatment

Interpretation of culturable airborne mold spore analysis is different from total mold spore counts because not all mold species can grow in culture medium; therefore, only common mold types are compared. Furthermore, this analysis allowed us to identify to the genus level what was assigned by the total airborne sampling method as *Penicillium/Aspergillus* type. It was determined that *Penicillium/Aspergillus* type mold spores were predominantly *Penicillium.* Measured culturable indoor concentrations of *Penicillium* were higher than those measured outdoor. *Penicillium* concentrations in the shower and locker areas represented 97 and 93% of the culturable fungal load, respectively. No *Penicillium* was measured outdoors. These results are consistent with total fungal spore concentration analysis, which indicated an indoor *Penicillium* source. See Table 3 for complete results.

Culturable fungi after treatment

When compared to pre-treatment concentrations, measured airborne culturable *Penicillium* spores were reduced 100 and 95% in the shower area and locker area, respectively. However, an increase in *Cladosporium* concentrations in both indoor areas was measured. This can be explained by the influx of outdoor spores that would have resulted once the facility was vented following ozone treatment. See Table 4 for complete results.

Surface Bacteria

2. *Surface Total Heterotrophic Bacteria:*
   A total of three surface samples were collected for the determination of total heterotrophic bacteria (THB) before and after ozone treatment. Two composite swab samples were collected using a template from a surface of approximately 250 cm^2 in the shower area and in the locker area. The locker area sample included lavatory handles, soap dispenser and door knobs in the restrooms. The same areas were measured after ozone treatment. A field blank swab sample was collected for analysis. See Table 5 for complete results.

   THB concentrations before and after ozone treatment were 80,000 and 40 Colony Forming Units/100 cm^2 (CFU/100 cm^2) respectively in the shower area. This signifies a reduction in surface THB of 99.5 % following ozone treatment. The second sample (collected in the locker area) is believed to have been contaminated, thereby making data interpretation on this sample difficult. See Table 5 for complete results.

3. *Additional Observations:*
   a. No indication of visible mold was observed in the surveyed areas.
   b. One ozone generator was placed in the shower area. Another two generators were placed in the locker area.
   c. The treated area was contained by sealing openings, and vents with duct tape.

CONCLUSIONS:

Bioaerosol measurements indicated the presence of a fungal source, which was determined to be predominantly *Penicillium,* in the men's locker room. This finding was consistently supported by both, total and culturable airborne fungi results. Likewise, elevated concentrations of total heterotrophic bacteria were measured on all sampled surfaces.

Measured total airborne fungal spores and culturable fungi concentrations after treatment with high ozone concentrations were consistently reduced. Reduction of culturable fungi concentrations ranged from 95-100%, while total fungal spores ranged from 38-97%. Reduction of total spores concentrations in the locker area was notably smaller (38%). Introduction of outdoor air to assist in exhausting ozone from the locker area likely resulted in the smaller spore reduction in that area.

After ozone treatment, total heterotrophic bacteria (THB) concentration was dramatically decreased (99.5%) in the shower area. However, it is believed that the post-treatment sample obtained in the locker area was contaminated as a ten-fold increase in THB concentration was

measured. The basis for stating that this sample was contaminated hinges on whole of the sampling data, which indicates a reduction of fungal and bacterial concentrations following ozone application in all other samples.

In summary, results obtained during this survey indicate that the treatment was effective in reducing microbial contamination. Furthermore, the largest reduction in total airborne *Penicillium* spores in the shower area indicates that cellular structures were not only inactivated but physically destroyed. The efficacy of the treatment remains to be evaluated with better certainty throughout a more comprehensive assessment. This assessment would include determining variability of microbial concentrations with respect to ozone levels. A summary of *all* results can be found in Table 7 of this report.

ENVIRONMENTAL HEALTH STANDARDS:

Final interpretation and statement of building containing elevated mold and bacterial levels is dependent on individual species recovered indoors, and on site-specific outdoor or comparison area concentrations for each species. Due to the well-documented extreme variability in individual response, medical professionals, especially those with a specialty in allergy and immunology, should always be contacted for a consultation if there is a report of symptoms that could possibly be related to fungal exposure. Bacterial exposure may pose infectious-acquired disease risks, which are also associated with the specific bacterial type.

SURVEY STRATEGIES AND PROCEDURES:

Bioaerosols were collected using spore-trapping media (Allergenco-D® Posi-Track cassettes) developed by Environmental Monitoring Systems. The samples were collected at a flow rate of 15 liters per minute, as calibrated with the Bio-Pump™ Flow Meter. Samples were collected using a Zefon® Bio-pump™ and analyzed microscopically by Quantus Analytical, LLC (AIHA EMPAT# 180253).

Air samples for culturable fungi were collected by drawing air through a Aerotech 6 single-stage viable samples at a flow rate of 28 Lpm. Sampled air was drawn through a 400-hole stage onto a Malt Extract Auger (MEA). Samples were analyzed at Quantus Analytical, LLC. Positive-hole correction was included in the calculation to adjust colony counts from a 400-hole impactor for the possibility of collecting multiple particles through a hole.

Composite samples were collected using swabs in selected areas. Samples were collected by wiping an area with a sterile swab using a 100 cm^2 template. New gloves and template were used every time a bacterial sample was collected. Samples were analyzed for Heterotrophic Counts Plate by EMLab P&K.

Submitted by:
Javier Ortega, MS
Senior Environmental Toxicologist

Reviewed by
Melissa M. Osborne, CIH, MS
Senior Industrial Hygienist

Report Attachments:
Data Tables (7)
Photo Documentation

This report is based upon information supplied by the customer and/or on the conditions we observed at the time of the visit and/or based on the samples taken and analyzed. The report may not list all unsafe conditions and practices; others may exist. No representation is made or intended that implementation of any recommendation provided rectify every issue relative to water incursion, mold proliferation, or poor indoor air quality. Surveys and recommendations are advisory and are designed to assist our customers in the management of their own air quality issues. The responsibility for implementing any recommendations is the customers.

Table 1
Total Airborne Fungal Analysis
Before Treatment

Sample Volume (L):	75			75		
Sample Number:	00144703			00129155		
Sample Location:	Shower Area			Locker Area		
Fungal Spore Type	Raw Count	Spores/m3	% of Total	Raw Count	Spores/m3	% of Total
Alternaria sp.	0	0	0.0	1	13	0.4
Aspergillus/Penicillium type	1,258	16,774	98.2	210	2,800	86.8
Basidiospores	13	172	1.0	15	200	6.2
Cladosporium sp.	6	86	0.5	10	133	4.1
Hyphal fragments	0	0	0.0	5	67	2.1
Smuts/Myxomycetes/Periconia	3	40	0.2	1	13	0.4
Spegganzinia sp.	1	13	0.1			
Totals	1,281	17,086	100	242	3,227	100
Background Debris	3			3		

Table 1 (continued)
Total Airborne Fungal Analysis
Before Treatment

Sample Volume (L):	75			75		
Sample Number:	00129837					
Sample Location:	Outdoor					
Fungal Spore Type	Raw Count	Spores/m3	% of Total	Raw Count	Spores/m3	% of Total
Alternaria sp.	8	107	2.5			
Ascospores	65	871	20.7			
Aspergillus/Penicillium type	12	163	3.9			
Basidiospores	118	1,578	37.5			
Cladosporium sp.	90	1,197	28.4			
Curvularia sp.	1	13	0.3			
Fusicladium	2	27	0.6			
Hyphal fragments	4	54	1.3			
Smuts/Myxomycetes/Periconia	10	133	3.2			
Torula sp.	5	67	1.6			
Totals	316	4,211	100	0	0	NaN
Background Debris	2			NA		

Table 2
Total Airborne Fungal Analysis
After Treatment

Sample Volume (L):	75			75		
Sample Number:	00137717			00137751		
Sample Location:	Outdoor			Showers		
Fungal Spore Type	Raw Count	Spores/m3	% of Total	Raw Count	Spores/m3	% of Total
Alternaria sp.	4	53	0.5	1	13	0.2
Ascospores	100	1,333	11.9	33	435	7.3
Aspergillus/Penicillium type	15	200	1.8	12	163	2.7
Basidiospores	315	4,200	37.5	143	1,905	31.9
Cladosporium sp.	355	4,733	42.3	237	3,156	52.8
Epicoccum sp.	3	40	0.4			
Hyphal fragments	5	67	0.6			
Oidium/Peronospora type	1	13	0.1			
Pithomyces sp.	0	0	0.0	1	13	0.2
Smuts/Myxomycetes/Periconia	40	533	4.8	21	280	4.7
Torula sp.	1	13	0.1	1	13	0.2
Totals	839	11,187	100	448	5,980	100
Background Debris	3			2		

Table 2 (continued)
Total Airborne Fungal Analysis
After Treatment

Sample Volume (L):	75			75		
Sample Number:	00138293					
Sample Location:	Lockers					
Fungal Spore Type	Raw Count	Spores/m3	% of Total	Raw Count	Spores/m3	% of Total
Alternaria sp.	1	13	0.2			
Ascospores	10	133	1.7			
Aspergillus/Penicillium type	80	1,067	13.2			
Basidiospores	155	2,067	25.6			
Cladosporium sp.	330	4,400	54.5			
Epicoccum sp.	1	13	0.2			
Hyphal fragments	10	133	1.7			
Pithomyces sp.	1	13	0.2			
Smuts/Myxomycetes/Periconia	17	227	2.8			
Totals	605	8,067	100	0	0	NaN
Background Debris	3			NA		

Table 3
Airborne Culturable Fungi
Before Treatment

Sample Volume (L)	141.5		141.5	
Sample ID	VM-1		VM-2	
Location Description	Shower Area		Locker Area	
Spore Type	Raw Count	CFU*/m^3	Raw Count	CFU*/m^3
Alternaria	-	-	-	-
Aureobasidium	-	-	-	-
Aspergillus	-	-	-	-
Basidiomycetes	-	-	-	-
Chaetomium	-	-	-	-
Cladosporium	12	84	7	49
Curvularia	-	-	-	-
Epicoccum	-	-	-	-
Fusarium	-	-	-	-
Non-Sporulating Fungi	-	-	-	-
Paecilomyces	-	-	-	-
Penicillium	361	2,551	91	643
Rhyzopus	-	-	-	-
Stachybotrys	-	-	-	-
Ulocladium	-	-	-	-
Yeasts	-	-	-	-
Total Spores	**373**	**2,635**	**98**	**692**

Table 3 (continued)
Airborne Culturable Fungi
Before Treatment

Sample Volume (L)	141.5		141.5	
Sample ID	VM-3			
Location Description	Outdoor			
Spore Type	Raw Count	CFU*/m^3	Raw Count	CFU*/m^3
Alternaria	-	-	-	-
Aureobasidium	-	-	-	-
Aspergillus	-	-	-	-
Basidiomycetes		-		
Chaetomium	-	-	-	-
Cladosporium	73	515	-	-
Curvularia	-	-	-	-
Epicoccum	-	-	-	-
Fusarium	-	-	-	-
Non-Sporulating Fungi	4	28	-	-
Paecilomyces	-	-	-	-
Penicillium	-	-	-	-
Rhyzopus	-	-	-	-
Stachybotrys	-	-	-	-
Ulocladium	-	-	-	-
Yeasts	-	-	-	-
Total Spores	**77**	**543**	**-**	**-**

Table 4
Airborne Culturable Fungi
After Treatment

Sample Volume (L)	141.5		141.5	
Sample ID	VM-11		VM-12	
Location Description	Outdoor		Showers	
Spore Type	Raw Count	CFU*/m^3	Raw Count	CFU*/m^3
Alternaria	-	-	1	7
Aureobasidium	-	-	-	-
Aspergillus	-	-	-	-
Basidiomycetes	-	-	-	-
Chaetomium	-	-	-	-
Cladosporium	1,010	7,137	79	558
Curvularia	-	-	-	-
Epicoccum	-	-	-	-
Fusarium	-	-	-	-
Non-Sporulating Fungi	3	21	2	14
Paecilomyces	-	-	-	-
Penicillium	4	28	-	-
Rhyzopus	-	-	-	-
Stachybotrys	-	-	-	-
Ulocladium	-	-	-	-
Yeasts	-	-	-	-
Total Spores	**1,017**	**7,186**	**82**	**579**

Table 4 (continued)
Airborne Culturable Fungi
After Treatment

Sample Volume (L)	141.5		141.5	
Sample ID	VM-13			
Location Description	Lockers Area			
Spore Type	Raw Count	CFU*/m^3	Raw Count	CFU*/m^3
Alternaria	-	-	-	-
Aureobasidium	-	-	-	-
Aspergillus	-	-	-	-
Basidiomycetes	-	-	-	-
Chaetomium	-	-	-	-
Cladosporium	117	826	-	-
Curvularia	-	-	-	-
Epicoccum	-	-	-	-
Fusarium	-	-	-	-
Non-Sporulating Fungi	3	21	-	-
Paecilomyces	-	-	-	-
Penicillium	6	42	-	-
Rhyzopus	-	-	-	-
Stachybotrys	-	-	-	-
Ulocladium	-	-	-	-
Yeasts	-	-	-	-
Total Spores	**126**	**889**	**-**	**-**

Table 5
Total Heterotrophic Bacteria
Before treatment

HETEROTROPHIC PLATE COUNT REPORT

Location:	SB-1: Pre treatment assessment	SB-2: Pre treatment assessment	SB-3: Pre treatment assessment
Comments (see below)	None	None	None
Lab ID-Version‡:	1826170-1	1826171-1	1826172-1
Sample Type	Swab sample	Swab sample	Swab sample
Method	Spread Plate: SM 9215C	Spread Plate: SM 9215C	Spread Plate: SM 9215C
Dilution	Plate Count Agar 1:1000	Plate Count Agar 1:10000	Plate Count Agar 1:10
Setup Time	04/28/2008 at 12:30 PM	04/28/2008 at 12:30 PM	04/28/2008 at 12:30 PM
Incubation Info	164 hrs at 23 +- 3C	164 hrs at 23 +- 3C	164 hrs at 23 +- 3C
Reporting Unit	1 swab	1 swab	1 swab
CFU/Reporting Unit	200,000	1,100,000	10

Comments:

Table 6
Total Heterotrophic Bacteria
After treatment

HETEROTROPHIC PLATE COUNT REPORT

Location:	SB-11: Post treatment assessment	SB-12: Post treatment assessment	SB-13: Post treatment assessment
Comments (see below)	None	None	None
Lab ID-Version‡:	1826173-1	1826174-1	1826175-1
Sample Type	Swab sample	Swab sample	Swab sample
Method	Spread Plate: SM 9215C	Spread Plate: SM 9215C	Spread Plate: SM 9215C
Dilution	Plate Count Agar 1:10	Plate Count Agar 1:10000	Plate Count Agar 1:10
Setup Time	04/28/2008 at 12:30 PM	04/28/2008 at 12:30 PM	04/28/2008 at 12:30 PM
Incubation Info	164 hrs at 23 +- 3C	164 hrs at 23 +- 3C	164 hrs at 23 +- 3C
Reporting Unit	1 swab	1 swab	1 swab
CFU/Reporting Unit	100	13,000,000 (est.*)	< 10

Comments:

* Estimated result

‡ A "Version" greater than 1 indicates amended data.

Table 7
Summary of Fungal and Bacterial Findings

Measurement	Location	Pre-treatment	Post-treatment	Results
Total Airborne Fungal Spores	Shower Area	16,774 spores/m^3 *	163 spores/m^3	97% decrease
Total Airborne Fungal Spores	Locker Area	2,800 spores/m^3 *	1,067 spores/m^3	38% decrease
Airborne Culturable Fungi	Shower Area	2,551 cfu/m^3§	0 cfu/m^3	100% decrease
Airborne Culturable Fungi	Locker Area	91 cfu/m^3§	42 cfu/m^3	95 % decrease
Surface Total Bacteria (THB)	Shower Area	80,000 cfu/100 cm^2	40 cfu/100 cm^2	99.5 decrease

*Measured as *Aspergillus/Penicillium* type spores
§Measured as *Penicillium* colony forming units (cfu)
±Possibly cross-contaminated

Photo Documentation

Figure 1. Total and culturable fungi sampling in shower area.

Figure 2. Shower area wall

Figure 3. Shower area entry

Figure 4. Locker area

Figure 5. Restroom lavatory

Figure 6. Clean Air System's Team before treatment

Figure 7. Setting containment. Sealing air diffusers

Figure 8. Sampling total and culturable fungi outdoors