

# Microbial Decontamination of a 65-Room New Pharmaceutical Research Facility

Mark A. Czarneski

ClorDiSys Solutions, Inc., Lebanon, New Jersey

## Abstract

*The following article describes a case study about decontamination of a 65-room new animal research facility located in the Northeast. The decontamination took place during the cold winter month of January, and all equipment used to run the facility was in place prior to beginning the decontamination. This facility had an essential need for complete decontamination because a lot of its equipment was procured from other facilities and cross-contamination was a concern. Chlorine dioxide gas was used due to the inherent properties of a gas, such as excellent distribution and penetration which were required due to the numerous rooms in the facility. The target concentration was not reached, but since photometric measurement was utilized, the exposure was extended accordingly and the end result was successful. All biological indicators were eradicated, and no residues and no material degradation were observed.*

## Facility Description

The end-user of a new 65-room, 18,000 sq ft (180,000 cu ft / 5097 cu m) life science research facility required decontamination prior to the opening and occupying of the facility. The entire area was to be decontaminated prior to moving in the animals, since some of the equipment was previously used at another facility and the possibility of cross-contamination needed to be eliminated.

Chlorine dioxide gas was used to fully decontaminate the area. The facility contained a variety of rooms such as a chemistry lab, animal holding rooms, procedure rooms, a cage wash room, bathrooms/showers, etc. No offices were located within the decontamination area. All the equipment needed to run the facility was in place prior to the decontamination. Various types of equipment, such as rodent racks and cages, bedding changing stations, biological safety cabinets, various plastics (for example, cages and water bottles), microscopes, video cameras, circuit-breaker panels, bathrooms and showers, smoke detectors, temperature and relative humidity (RH) sensors, as well as various analytical and electronic equipment used in a typical chemistry lab, were stored within the facility. All surfaces in the area were clean and non-porous, including stainless steel, epoxy-painted walls, solid flooring, painted steel cabinets, and plastic light fixtures. The facility was cleaned prior to the decontamination.

## Background Information: Determine What Method to Use

To perform a facility decontamination, a choice had to be made to determine which decontamination agent to utilize. In this case three were considered: formaldehyde gas, vapor phase hydrogen peroxide (VPHP), and chlorine dioxide (CD) gas. All were known to be effective decontaminants for spore and non-spore forming bacteria under ideal laboratory conditions (i.e., clean flat surfaces lacking porous materials or potential dead-legs with which fumigant penetration might be retarded).

### Formaldehyde Gas

Formaldehyde gas is the agent that is probably used most frequently when compared to CD gas and VPHP. It is used most often because it is effective (gets good kill) (Wickramanayake, 1990), is a gas (gets distribution and penetration), and the equipment necessary to perform a decontamination (hot plate) is low in cost (\$19.99 at local department stores). The negative issues for formaldehyde are that it is listed as a carcinogen by the International Agency for Research on Cancer (IARC, 2004), and it requires a post-exposure cleanup.

The first step in performing a formaldehyde decontamination is to raise the humidity to 65-75%. Once this is accomplished, the paraformaldehyde powder is heated up on hot plates. Typically, 0.3g of paraformaldehyde per cubic foot of room volume is decontaminated (NSF Annex G, 2008). Once the formaldehyde gas is released, exposure time follows which is typically 12 hours. After this, neutralization takes place by heating up ammonia bicarbonate or ammonium carbonate to generate an ammonia vapor. This ammonia vapor then neutralizes the formaldehyde gas and creates a relatively safe byproduct called methenamine (Luftman, 2005). This byproduct or residue was the main reason formaldehyde was rejected as a choice, since the residue that is created when neutralizing the formaldehyde gas in such a large facility was determined to be too problematic. In addition, due to formaldehyde re-polymerizing back to paraformaldehyde on cold surfaces (Ackland et al., 1980), the perceived toxicity was a concern if cleanup was not performed effectively.

### Hydrogen Peroxide Vapor (VPHP)

Hydrogen peroxide vapor has been available since the 1980s and is gaining favor over formaldehyde gas,

since VPHP is non-carcinogenic and does not require a post-exposure wipe-down. It has been used primarily at pharmaceutical companies to decontaminate isolators and other small chambers. To generate the vapor, 35% liquid hydrogen peroxide is flash vaporized and delivered to the target chamber. This vapor has natural tendencies to condense on surfaces since at room temperatures hydrogen peroxide is a liquid. This condensation has better antimicrobial properties (Watling et al., 2002) since as it condenses its concentration increases (35-78%) (Hultman et al., 2007). While the antimicrobial activities may be enhanced due to condensation, the distribution and penetration (which are critical in any decontamination) are limited due to the nature of a vapor (Herd, 2005a; Shearrer, 2006). Additionally, condensation has also been demonstrated to cause corrosion on surfaces (Hultman et al., 2007; Malmborg et al., 2001). These two reasons (lack of distribution and corrosion) are why VPHP was thought to be too limiting to use in this facility and was not selected. When performing large-scale decontaminations using VPHP, typically the areas are segregated into smaller areas to reduce the number of generators required and to aid the distribution (EPA/600/R-05/036, 2005a; Herd et al., 2005b). This leads to a potentially negative issue when segregating areas—the probability of recontaminating an area that was just decontaminated. When moving equipment from the clean area to the next area to be decontaminated; the user may inadvertently contaminate a previously clean area. The more divisions it takes to decontaminate large volumes, the higher the probability this may occur. Additionally, segregating the areas creates more work, takes more time, and thereby increases costs.

### Chlorine Dioxide (CD) Gas

Chlorine dioxide gas is a relative newcomer to the decontamination field. Chlorine dioxide in liquid has been recognized as a powerful high-level liquid disinfectant for a long time and is used for water treatment and food processing. In the late 1990s, gaseous chlorine dioxide's decontamination ability was recognized and became commercially available around 2001. Chlorine dioxide gas has demonstrated its significant abilities to decontaminate with minimal external considerations (CD does not condense, temperature does not affect it, and paper products or galvanized steel do not cause it to break down as with VPHP (Carlsen, 2005). CD was used in most of the anthrax-contaminated facilities in Washington, DC, the post office in Hamilton, NJ, and the news media offices in Florida (EPA/600/R-05/036, 2005b).

Chlorine dioxide gas has more efficacy than the liquid and none of the issues that have long been associated with the (acidic sodium chlorite) solution. Gaseous CD has also been shown to attain 7.2 log reduction compared to a 3.6 log reduction for liquid CD at equal concentrations and exposure times (Han et al., 2000).

Corrosiveness is an issue with CD, depending on the generation method. When generated as a gas (no liquids), the acidic sodium chlorite solution is not present.

One further decontamination note is that all decontamination agents have drawbacks. Both VPHP (1.78V oxidation potential) and CD (0.95V oxidation potential) are oxidizers (Wintner et al., 2005), formaldehyde while gentle on materials is a carcinogen, ethylene oxide has the best penetration but is explosive (NIOSH et al., 1994) and methyl bromide is classified as an ozone-depleting substance (EPA, 1993).

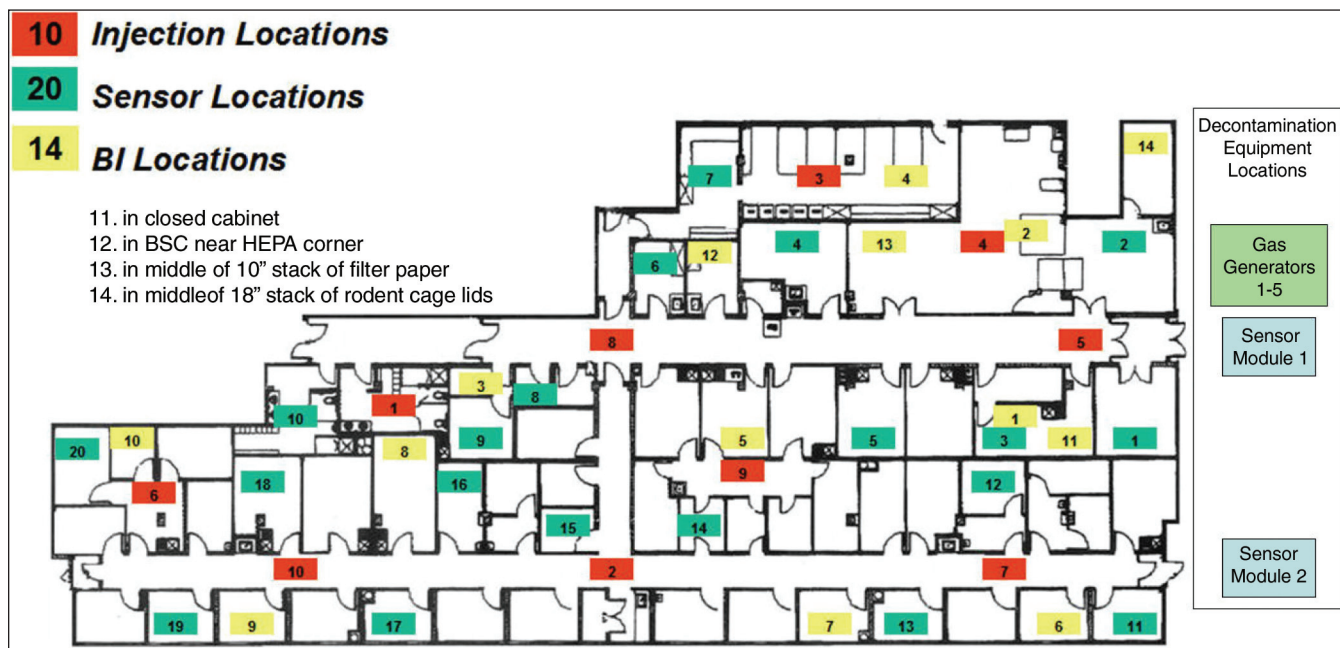
The benefits of chlorine dioxide are that, like formaldehyde, it is a true gas at room temperature and has the inherent properties of good distribution and excellent penetration. It is not affected by temperatures, does not condense or fall out on surfaces, is non-carcinogenic, and requires no post-exposure wipedown. Since the facility is complex and has many rooms, it was decided to use a gaseous agent to get the proper distribution and penetration.

Chlorine Dioxide Properties
• Yellow-Green Gas
• Water Soluble
• Boiling Point 11°C
• Tri-atomic Molecule
• Molecular Weight 67.5

### Materials and Methods

- 5 – ClorDiSys Solutions, Inc., Lebanon, NJ, Cloridox-Manual – chlorine dioxide gas generators.
- 2 – ClorDiSys Solutions, Inc., Lebanon, NJ, Cloridox-EMS – chlorine dioxide (CD) measurement system (0-50 mg/L)
- 1 – Interscan Corporation, Chatsworth, CA, 4330-1 – low level CD sensor (0-199.9ppm)
- 5 – Extech, Waltham, MA, 445814 – Relative Humidity Sensors
- 70 – 12" plastic distribution fans
- 6 – 20" box distribution fans
- 20 – ClorDiSys Solutions, Inc., Lebanon, NJ, Steam Fast, STMGEN-SF-001 – steam humidifiers
- 14 – SGM, Bozeman, MT, Strip Biological Indicator ACD/6 – wrapped in a tyvek/mylar pouch, *Bacillus atrophaeus* (ATCC 9372) spores strips on paper carrier (population 2.2 x 10<sup>6</sup>)
- Releasat Media RM/100 – Modified Soybean Casein Digest Broth, incubating media
- 3/8" polyethylene tubing (red) – gas inject
- 1/4" polyethylene tubing (green) – sample tubing
- Plastic sheeting (6 mil)
- Duct tape

**Figure 1**  
Facility Layout



## Day 1: Site Preparation

The total site preparation was a 1-day process for three people. To prepare the site for decontamination several things had to be done. The first item was to seal the facility. This step is the same for all methods; independent of which agent is used. The chamber must be sealed to contain the decontaminating agent, whether formaldehyde gas, VPHP, or CD gas is used. The HVAC system was shut down since the entire facility was being decontaminated. If only individual rooms were decontaminated, then only those rooms would have the dampers closed. In addition to shutting down the HVAC, the supply inlet and exhaust outlet in the mechanical room on the roof (four stories above the facility) were covered and taped with plastic sheeting. Even though the supply inlet and exhaust out were four floors away, they had to be sealed with plastic because gases have excellent distribution and do reach these areas. All of the entry doors (except one used for entry and exit prior to the decontamination) were sealed with duct tape. After this, the gas inject tubing and gas sample tubing were set up (see Figure 1 for placement). The gas generators were located outside the decontamination area and 3/8" tubing was run from the gas generators to the gas inject locations (10 sites). The generators were located outside the decontamination area as a safety precaution. If equipment issues develop during the process, in this way the process is easy to shutdown. If equipment is located inside the area being decontaminated, shutting down the equipment can become a safety concern. After the gas injection tubing was run, the sensor module tubing was

next. There were 10 sensing points for Sensor Module 1 and 10 points for Sensor Module 2. Tubing (1/4") was run from the sensor modules to each sensor location (20 in total). A valve manifold was used to multiplex the 10 sensing points to one of the two sensor modules. The fans and humidifiers were then placed around the facility, basically one 12" fan per room and several of the box fans in the hallways. These were used to aid the distribution of the gas and humidity. Fans were typically placed at the entry to each room to provide a turbulent environment. Fans were not in oscillating mode.

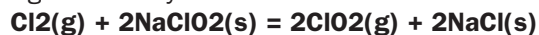
## Day 2: Decontamination

On the second day, and once all the decontamination equipment was in place and the facility was sealed, the biological indicators (BIs) were placed around the facility. *B. Atrophaeus* BI was chosen as the BI because of its resistance. This resistance was demonstrated in several sources. Jeng performed studies that showed that *B. subtilis* var. *niger* (now renamed to *B. Atrophaeus*) was more resistant (Jeng et al., 1990) to typical BIs. Czarneski also demonstrated a greater resistance of *B. Atrophaeus* to CD gas (Czarneski et al., 2008), and Luftman chose *B. Atrophaeus* over *G. Stearothermophilus* (Luftman et al., 2008) in the validation of CD gas. Ten BIs were placed in rooms that did not have gas sampling or injection points to demonstrate distribution (see Figure 1 for room placement). Each of the 10 BIs was placed in the rear of the rooms in the farthest corner from the hallway. This place was chosen since gas was injected into the hallways. An additional four were placed in areas that were deemed

extremely difficult to decontaminate and where the facility people did NOT expect the BIs to be killed. These were placed in a closed cabinet, inside a BSC near HEPA corner, in the middle of a 10" stack of filter paper, and in the middle of an 18" stack of rodent cage lids. Typically cabinets are opened and stacks of items are spread out to allow for the decontamination agent access. Biological indicators were not a requirement for this particular event, so minimal BIs were used to validate the process. The process used is registered with the US-EPA (EPA #80802-1), and if certain conditions are met, then biological efficacy is ensured. The validated photometric measurement ensures proper concentrations are met for 6-log sporicidal kill. Biological indicators are placed as a secondary verification.

After the BI placement, the steam generators were energized to raise the humidity. Twenty steam generators were placed throughout the facility, and RH was measured in each room using a hand-held RH sensor. During this time it was noted that the humidity was not increasing to the desired target of 65-70%. Readings taken varied from 50-60%. Upon inspection, it was found that several exhaust plenums were not sealed, thus allowing the humidity to escape. Once this was corrected by seal-

ing the exhaust ports, the humidity quickly rose to the minimum of 65%. Each room was checked and verified to be at a minimum of 65% RH. Raising the humidity gives the ability to safely check that the HVAC system and facility are properly sealed and shut down. Noting that the humidity level had not increased as expected caused an inspection to locate and close the open exhaust plenums. After raising the RH to a minimum of 65%, it was allowed to sit and stabilize for 30 minutes. During humidification all but one doorway was sealed. This allowed the monitoring of individual rooms for proper RH levels. If the RH in any room was too low, humidifiers were moved or rotated to bring the low RH room to the desired level. After all the rooms were at the proper RH level for a minimum of 30 minutes, the steam generators were removed. At this point the facility and BIs had the necessary conditioning, and upon exiting the area, no condensation on surfaces was observed. Finally, the last door was sealed and the 2% chlorine cylinders were opened so that the process to generate the CD gas began. Gas flowed at a slow rate of 20 liters per minute through cartridges containing mostly sodium chlorite. CD gas is generated by the reaction:



**Table 1**  
Readings for Sensor Module 1

Time	Sensor Location										
	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8	Series 9	Series 10	
12:20	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	Charge
1:05	0.3	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.4	
2:00	0.4	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.6	
2:45	0.5	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.6	0.7	
3:25	0.6	0.7	0.6	0.7	0.6	0.7	0.7	0.7	0.7	0.8	
4:00	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.9	
4:45	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	
5:35	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.6	
6:15	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	
7:00	0.7	0.7	0.7	0.6	0.7	0.6	0.5	0.6	0.6	0.6	
7:35	0.2	0.2	0.2	0.1	0.1	0	0	0	0	0.1	Aeration
7:50	0	0	0.1	0	0	0	0	0	0	0	Aeration
8:00	0	0	0	0	0	0	0	0	0	0	Aeration
avg mg/L	0.567	0.656	0.589	0.622	0.6	0.622	0.611	0.622	0.589	0.656	
avg ppm	205.1	237.3	213.2	225.2	217.2	225.2	221.2	225.2	213.2	237.3	
ppm hrs	1231	1424	1279	1351	1303	1351	1327	1351	1279	1424	
										1332	Avg ppm hrs reading 1-10
										222	Avg ppm readings 1-10

The gas from the five generators was distributed to 10 injection points. Each generator held two cartridges that converted the low-level chlorine gas to chlorine dioxide gas. Once the CD gas generation was started, it was monitored (see Tables 1 and 2 for readings).

## Results

A CD gas concentration of 1 mg/L was targeted. As demonstrated by the readings (Tables 1 and 2), this was not achieved because three leaks were detected. An odor was detected (odor threshold of CD is 0.1 ppm (ACGIH, 2001) in the mechanical room above points series 18 and 19, and the other leaks detected were up the exhaust stack. There was difficulty sealing the exhaust grills on the roof to the roof surface, and in the mechanical room the supply inlet was not adequately taped. These leaks caused an inability to meet target concentration but were too small to cause safety issues or concerns. There was a slight buildup of concentration in the mechanical room, but the outside door was left open to draw the gas out. One benefit was that CD gas is unique

in that it is detectable by odor. Had this not been the case, the leak could have continued to build up to unsafe concentrations in this closed area. The entire area surrounding the facility was periodically scanned with a low-level sensor and no other leaks were detected. This is a precautionary measure that should be done regardless of what decontamination method is utilized and should be performed periodically during the charge or exposure phases.

Since the target concentration was not reached, exposure time was impacted. The target concentration of 1mg/L or 362ppm has a typical exposure time of 2 hours, which equates to (362 ppm \* 2 hours) = 724 ppm-hrs. Since the 1mg/L was not reached, the exposure time had to be extended. From the readings taken, the exposure time was extended from 2 hours to 6 hours. This was determined by accruing the ppm-hrs every hour. When the minimum of 720 ppm-hrs was exceeded, the exposure was terminated.

For example, sensor reading series 1—at 2 pm there was an average concentration of 0.35 mg/L for 1 hour or 126.7 ppm-hrs:

$$(0.35 \text{ mg/L} * 362 \text{ PPM} * 1 \text{ hour}) = 126.7 \text{ ppm-hrs}$$

**Table 2**  
Readings for Sensor Module 2

Time	Sensor Location										
	Series 11	Series 12	Series 13	Series 14	Series 15	Series 16	Series 17	Series 18	Series 19	Series 20	
12:25	0	0	0.1	0.1	0	0	0	0	0	0	Charge
1:10	0.4	0.4	0.5	0.4	0.3	0.3	0.4	0.3	0.3	0.3	
2:15	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.3	0.3	0.5	
2:50	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.3	0.3	0.5	
3:35	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.4	0.4	0.6	
4:00	0.8	0.7	0.8	0.7	0.6	0.6	0.6	0.4	0.5	0.6	
4:50	0.9	0.8	0.8	0.6	0.5	0.5	0.5	0.4	0.4	0.6	
5:35	0.9	0.9	0.8	0.6	0.5	0.5	0.5	0.4	0.4	0.6	
6:15	0.9	0.9	0.9	0.6	0.5	0.5	0.6	0.4	0.5	0.6	
7:00	0.9	0.8	0.8	0.6	0.5	0.6	0.6	0.5	0.5	0.5	
7:35	0	0.1	0	0	0	0	0	0	0.1	0	Aeration
7:50	0	0	0	0	0	0	0	0	0	0	Aeration
avg mg/L	0.733	0.7	0.711	0.578	0.489	0.511	0.533	0.378	0.4	0.533	
avg ppm	265.5	253.4	257.4	209.2	177	185	193.1	136.8	144.8	193.1	
ppm hrs	1593	1520	1545	1255	1062	1110	1158	820.5	868.8	1158	
										1209	Avg ppm hrs reading 11-20
										201.5	Avg ppm readings 11-20
										1271	Total Avg ppm hrs
										211.8	Total Avg ppm readings 1-20

The ppm-hrs are then accrued each hour until the lowest reading has exceeded 720 ppm-hrs. In this case study that low reading was sensor point 18. Once the desired exposure or minimum contact time was completed, aeration was initiated.

Aeration was initiated by starting the exhaust system and removing the plastic covering the exhaust grills. Next, the supply was started and the plastic covering the supply inlets was removed. By starting aeration in this way, possible worker exposure to CD is minimized. If the supply was started first, then the facility would be pressurized and force the gas into the adjoining areas. Within 30 minutes most of the readings were at 0.0 mg/L to 0.1 mg/L and a few at 0.2 mg/L. At 50 minutes all the readings on the CD gas sensor module were 0.0 mg/L and at 60 minutes the concentration was at or below 0.1 ppm. The current Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for chlorine dioxide is 0.1 ppm as an 8-hour time-weighted average (TWA) concentration [29 CFR 1910.1000, Table Z-1]. Using the low-level sensor, no CD gas was measured on the roof or at ground level, although a slight odor was detected on the roof. Sixty minutes after aeration was started, the facility was entered using proper gowning techniques, and the fans and injection and sampling tubing were removed. The facility was then immediately ready for occupation by workers and research animals.

## Discussion

In this new facility, full decontamination of the building and all the equipment it contained was imperative before running the facility. This was essential since cross-contamination can have a real or perceived impact on experiments and the results of any endeavors within the facility. This was also critical since much of the equipment came from other facilities. When decontaminating a facility or any space with any agent, three requirements were identified (Czarneski, 2007):

1. Good and complete distribution
2. Good and total penetration
3. Sufficient contact time at specified concentration

Using these requirements, the facility layout, and the amount of equipment in place at the time of decontamination, a choice had to be made as to which agent to use. All agents that were considered work well with clean surfaces with no porous materials or difficult-to-reach areas, but this facility had all the equipment in place with many difficult-to-reach areas. Additionally, the agent chosen had to have superior distribution properties and leave no residues. Eylath et al. (2003b) measured post-exposure rinses of 304SS coupons in water for injection (WFI) and showed no residual CD as measured using an HPLC method for detection of chlorides. The methods that provide the best distribution and penetration are formaldehyde gas and chlorine dioxide gas. Formaldehyde was eliminated due to the residues. If the sterilant does not

reach the surface, then it cannot kill what it does not reach. A third component to distribution and penetration is contact for a certain time. Contact time was also demonstrated by the photometric measurement. If these measurements had not been performed, the contact time would have been insufficient and the level of kill would not have reached the desired level. Based on these requirements, chlorine dioxide gas was chosen.

The targeted CD gas concentration was not reached as identified by the CD gas sensors. This measurement method analyzes a certain wavelength that gives an absorption measurement. This absorbance is then converted to mg/L for easy concentration monitoring. This process is used by a variety of governmental research organizations which have in turn validated the process (Shah et al., 2005). The benefit of utilizing this method is that it provides repeatable readings and does not saturate as compared to chemical-based measurements. Sample tubing is run from the sensor module to the point where the sample is taken. This distance can be more than 500 ft (150m). In this particular application the longest sample run was approximately 200 ft. The sample is pulled through the photometer by a small diaphragm pump and returned back to the gassing areas. The photometer provided real-time measurement of the sterilant concentration within the facility at 20 different locations. By using it, leakage issues were uncovered. This underlines why sterilant gas photometric measurement is important. If formaldehyde decontamination had been used, the low levels of the gas would not have been identified and the cycle could not be adjusted. By comparison, chemical-based sensors are notorious for getting saturated and taking time to read accurately or consistently after being saturated. This is why photometric measurement is significant. The CD photometric measurement is not affected by temperature, humidity, or high or low concentrations and therefore is a reliable measurement device.

Determining how long to extend the exposure time is achieved by summing up the exposure in ppm-hrs. As shown in Tables 1 and 2, the total ppm-hrs all exceeded the minimum of 720 ppm-hours which is typically targeted for a 6-log spore reduction. The value of 720 ppm-hours is based upon current published knowledge; for example, Luftman demonstrated a minimum of 5-log reduction at 400 ppm-hrs (Luftman et al., 2006) at a decontamination of a large animal hospital, and Czarneski demonstrated 6-log reduction in an isolator at 900 ppm-hrs (Czarneski et al., 2005). Eylath demonstrated various cycles in various applications (isolators and processing vessels). All cycles obtained a 6-log reduction of spores. The tests were run at various exposures ranging from 540 ppm-hrs to 600 ppm-hrs as well as 900 ppm-hrs in processing vessels (Eylath et al., 2003a). In isolators Eylath demonstrated 6-log reductions in exposures of 675 ppm-hrs to 1800 ppm-hrs (Eylath et al., 2003b). Additionally, a 4-log reduction was demonstrated

by Leo at a low exposure of 180 ppm-hrs (1mg/L for 30 min.) (Leo et al., 2005).

Based upon calculations done after the decontamination, the actual minimum concentration-hours was 820 ppm-hrs. With the desired ppm-hrs reached, the real success factor were the results of the BIs. The exposed BIs and one positive control BI were dropped in vials containing modified soybean casein digest broth. The BIs were then placed in an incubator set for 33°C. *B. Atrophaeus* spores have a recommended incubation temperature of 30-35°C and an incubation time of 7 days. After the 7 days of incubation, it was noted that all the vials, except for the positive control, had no color change. A color change from red/orange to yellow indicated growth or non-sterility. Only the positive control showed growth.

Also worth mentioning is that the area was free of CD gas within 1 hour of aeration and safe to enter. If formaldehyde gas had been used, the neutralization process would have required 2 hours, and once this was accomplished residues would require clean-up. If VPHP had been used, only one area would be decontaminated, since the facility would have been segregated into smaller areas. Additionally, the aeration would typically continue into the next day, at which point the equipment would be moved from the clean area to the next area and the setup/decontaminate/aerate process would start again until all areas were completed. It was estimated that the area would be broken down into 3-5 areas and the process would have taken more than 1 week.

## Conclusion

One unknown benefit of chlorine dioxide was uncovered during this case study. During the conditioning phase (raising the RH), a few exhaust grills were discovered that were not sealed. This was discovered when the RH levels were not coming up to the desired 65-70%. If the first step had been to inject the sterilant, that agent would have leaked into the area and could have caused an issue. Since humidification was performed in a previous step, the problem was uncovered and no issue occurred.

The chlorine dioxide gas decontamination of the 65-room facility was a complete success. This was determined by gas distribution at proper levels, length of exposure at measured values, complete kill of all biological indicators, no physical residue, and no visible indication of material degradation on any of the metal-containing equipment left within the building, including the ventilated racks, plastic caging, BSCs, various electronics, etc. Also of note—there was no visible indication of effect to any electronics or measurement devices in the area. The success of CD was also demonstrated at low levels of CD (average concentration of 211.85 ppm); consequently, CD has proven itself to be a practical and effective method for decontaminating large facilities with minimal work, minimal time (2 days), and therefore lower costs compared to other methods.

## Acknowledgements

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## References

- ACGIH. (2001). American Conference of Governmental Industrial Hygienists threshold limit values (TLVs®) for chlorine dioxide.
- Ackland, N. R., Hinton, M. R., & Denmeade, K. R. (1980). Controlled formaldehyde fumigation system. *Applied and Environmental Microbiology*, 39(3), 480-487.
- Carlsen, T. (2005). *Use of HVAC systems in building decontamination*. S. Dun, J. Wood, & B. Martin (Eds). Presentation for Workshop on Decontamination, Cleanup, and Associated Issues for Sites Contaminated with Chemical, Biological, or Radiological Materials. Washington, DC: Office of Research and Development, U.S. Environmental Protection Agency. Contract No. EP-C-04-056.
- Czarneski, M. A., & Lorcheim, P. (2005). Isolator decontamination using chlorine dioxide gas. *Pharmaceutical Technology*, 29(4), 124-133.
- Czarneski, M. A., & Lorcheim, P. (2008). Validation of chlorine dioxide sterilization. In J. Agalloco & F. Carleton (Eds.), *Validation of pharmaceutical processes* (3rd ed.) (pp. 281-287). New York: Inform Healthcare, Inc.
- Czarneski, M. A. (2007). Selecting the right chemical agent for decontamination of rooms and chambers. *Applied Biosafety: Journal of the American Biological Safety Association*, 12(2), 85-92.
- EPA. (1993). Environmental Protection Agency, 40 CFR Part 82, *Protection of stratospheric ozone*, 58(236), 65018 (Rule) 8006.
- EPA/600/R-05/036. (March, 2005a). *Building decontamination alternatives, Section 5.2.4.2 Experience with field fumigation of buildings*. Washington, DC: U.S. Environmental Protection Agency National Homeland Security Research Center Office of Research and Development. Prepared by Science Applications International Corporation.
- EPA/600/R-05/036. (March, 2005b). *Building decontamination alternatives, Section 5.1.4.2 Experience with field fumigation of buildings*. Washington, DC: U.S. Environmental Protection Agency National Homeland Security Research Center Office of Research and Development. Prepared by Science Applications International Corporation.
- EPA #80802-1, U.S. Environmental Protection Agency, Office of Pesticide Programs, List A: EPA's Registered Antimicrobial Products Registered with the EPA as Sterilizers, January 9, 2009.
- Eylath, A. S., Madhogarhia, E. R., Lorcheim P., & Czarneski, M. A. (August, 2003a). Successful sterilization using chlorine dioxide gas: Part two—Cleaning process vessels. *BioProcess International*, 1(8), 54-56.
- Eylath, A., Wilson, D., Thatcher, D., & Pankau, A. (July, 2003b). Successful sterilization using chlorine dioxide gas: Part one—Sanitizing an aseptic fill isolator. *BioProcess International*, 1(7), 52-56.

- Han, Y., Sherman, D. M., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2000). The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157: H7 to green pepper surfaces. *Food Microbiology*, 17(5), 521-533.
- Herd, M. (2005a). *Hydrogen peroxide vapor for room/building decontamination following a chemical or biological agent attack: Overview of efficacy and practical issues*. Presentation for Workshop on Decontamination, Cleanup, and Associated Issues for Sites Contaminated with Chemical, Biological, or Radiological Materials. S. Dun, J. Wood, & B. Martin (Eds). Washington, DC: Office of Research and Development, U.S. Environmental Protection Agency. Contract No. EP-C-04-056.
- Herd, M. I., & Warner, A. (2005b). Hydrogen peroxide vapor biodecontamination of the Jackson Laboratory's new animal facility. *Animal Lab News*, 4(7), 31-39.
- Hultman, C., Hill, A., & McDonnell, G. (2007). The physical chemistry of decontamination with gaseous hydrogen peroxide. *Pharmaceutical Engineering*, 27(1), 22-32.
- International Agency for Research on Cancer. (June 30, 2004). Available at: [www.iarc.fr/ENG/Press\\_Releases/archives/pr153a.html](http://www.iarc.fr/ENG/Press_Releases/archives/pr153a.html)
- Jeng, D. K., & Woodworth, A. G. (1990). Chlorine dioxide gas sterilization under square-wave conditions. *Applied Environmental Microbiology*, 56, 514-519.
- Leo, F., Poisson, R., Sinclair, C. S., & Tallentire, A. (2005). Design, development, and qualification of a microbiological challenge facility to assess the effectiveness of BFS aseptic processing. *PDA Journal of Pharmaceutical Science and Technology*, 59(1), 33-48.
- Luftman, H. S. (2005). Neutralization of formaldehyde gas by ammonium bicarbonate and ammonium carbonate. *Applied Biosafety: Journal of the American Biological Safety Association*, 10(2), 101-106.
- Luftman, H. S., Regjts, M. A., Lorcheim, P., Czarneski, M. A., Boyle, T., Aceto, H., et al. (2006). Chlorine dioxide gas decontamination of large animal hospital intensive and neonatal care units. *Applied Biosafety: Journal of the American Biological Safety Association*, 11(3), 144-154.
- Luftman, H. S., & Regjts, M. A. (2008). *B. Atrophaeus* and *G. Stearothermophilus* biological indicators for chlorine dioxide gas decontamination. *Applied Biosafety: Journal of the American Biological Safety Association*, 13(3), 143-157.
- Malmborg, A., Wingren, M., Bonfield, P., & McDonnell, G. (2001). VHP takes its place in room decontamination. *CleanRooms*, 15(11) Available at: [http://cr.pennnet.com/articles/print\\_toc.cfm?p=15&v=15&i=11](http://cr.pennnet.com/articles/print_toc.cfm?p=15&v=15&i=11)
- National Sanitation Foundation (NSF). (2008). International Standard 49 Annex G.
- National Institute for Occupational Safety and Health (NIOSH) & Centers for Disease Control and Prevention (CDC). (1994). International Chemical Safety Cards, ethylene oxide. Available at: [www.cdc.gov/niosh/ipc sneng/neng0155.html](http://www.cdc.gov/niosh/ipc sneng/neng0155.html)
- Shah, S., Sickler, T., Smith, L., Wallace, L., & Rastogi, V. (2005). *Validation of photometric measurement of chlorine dioxide gas*. Timonium, MD: Scientific Conference on Chemical and Biological Defense Research.
- Shearrer, S. (2006). *Comparison of formaldehyde vs. VHP decontamination within operational BSL-4 laboratory at Southwest Foundation for Biomedical Research, San Antonio, Texas*. Boston: Presentation at 49th Annual Biological Safety Conference Program.
- Watling, R., & Parks, C. (2002). Theoretical analysis of the condensation of hydrogen peroxide gas and water vapour as used in surface decontamination. *Pharm SciTech*, 56(6), 291-299.
- Wickramanayake, G. B. (1990). Decontamination technologies for release from bioprocessing facilities—Part IV: Decontamination of equipment/surfaces. *Critical Reviews in Environmental Control*, 19(6), 481-513. [See Tables 4 and 6. The Wickramanayake article summarizes data previously published in the following sources, which were not reviewed for the present report: (1) Bovallius & Anas, 1977; (2) Caputo & Odlaug, 1983; and (3) Klein & De Forrest, 1983.]
- Wintner, B., Contino, A., & O'Neill, G. (2005). Chlorine dioxide—Part 1A: Versatile, high-value sterilant for the biopharmaceutical industry. *BioProcess International*, 3(11), 42-46.

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