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CASE STUDY:
Chlorine Dioxide Gas Decontamination of the University of Pennsylvania's George D. Widener Large Animal Hospital Intensive and Neonatal Care Units

After a salmonellosis outbreak, the 170,000 cubic foot C. Mahlon Kline building remained under quarantine until a successful decontamination program was implemented

The George D. Widener Large Animal Hospital (Figure 1) is one of the largest equine hospitals in the United States, serving nearly 6,000 patients yearly. This hospital also serves as an integral part of the University of Pennsylvania's School of Veterinary Medicine and is located at New Bolton Center, Kennett Square, PA.

A salmonellosis outbreak was detected in March 2004. During the following months, multiple cases of salmonellosis were found among the animal population and Salmonella Newport was identified as present in each of these cases. Following this, it was deemed necessary to quarantine the entire Widener Hospital in May 2004 until the adverse bacterial population could be brought down.

The contaminating agent was identified as a multi-drug resistant form of Salmonella Newport, a gram-negative, non-spore forming bacteria. Decontamination efforts were made throughout the Widener Hospital. Several attempts were made at decontaminating the facility (see Figure 2) using a broad-spectrum liquid disinfectant (Virkon S). This solution containing potassium peroxomonosulfate, sodium dodecyclbenzenesufonate, and sulfamic acid (Virkon-S), which is an AOAC approved oxidizing detergent sanitizer, was applied on most surfaces following manufacturer instructions. After each attempt bacterial populations were reduced at many locations, but the population persisted within the facility. After several failed attempts at controlling the Salmonella population within this facility, it was decided that a gas-phase space decontamination was the most advantageous route to a successful decontamination of the C. Mahlon Kline intensive care and neonatal care building.

Current Decontamination Methods
Current decontamination methods for rooms and buildings include manual spray and wipe or soaking techniques using liquid disinfecting solutions; formaldehyde gas; hydrogen peroxide vapor; and chlorine dioxide gas. Manual spray and wiping and formaldehyde gassing are by far the primary methods and each has significant drawbacks.

Manual spray and wiping methods are the least effective methods of decontamination. It is subject to missed areas because of human error. The personnel performing the decontamination must spray each and every surface with the decontaminating agent, allow the agent to remain on the surface for the specified amount of time without drying (typically 10-20 minutes), then wipe the surface. This is particularly difficult in hard to reach areas such as corners, the under side of HVAC grills, floor drains, beneath components, etc.

Sprayers, foggers, and misters are an improvement of the manual spray and wipe technique since you have removed the person from the process, but they are limited in their ability or coverage. These methods create small size droplets that are heavier than air and settle downwards when sprayed or injected. While they get coverage on the walls, they do not get coverage on the underside of components. Additionally, spraying in odd shaped rooms, as in the Widener building does not get complete or even coverage. Formaldehyde is a very effective method that has been used longer than all of the other "gassing" methods and is well characterized. It is effective against a broad range of organisms, is low cost, and can effectively reach all surfaces. The major concern with decontaminating rooms, buildings, and vessels with formaldehyde is that it is listed as a potential carcinogen by the EPA (U.S. Department of Health and Human Services Public Health Services, National Toxicology Program, 2002). As of June 2004, the International Agency for Research on Cancer classified formaldehyde as carcinogenic to humans (IARC, 2004). Furthermore formaldehyde requires additional steps: neutralization and manual wiping of the neutralization byproduct. A residue is commonly left after such treatment, consisting of polymerized formaldehyde (paraformaldehyde) and the neutralization product (methenamine). The removal of such a residue was considered challenging for this facility. There was additional concern that residual formaldehyde from off-gassing would also be problematic both because of its odor and its perceived toxicity. This required step increases the amount of time involved, amount of personnel involved, and the cost in labor and time. Furthermore, formaldehyde concentrations are not monitored during the process thereby not assuring complete coverage throughout the facility.

Vapor hydrogen peroxide is somewhat effective under ideal conditions but falls short in real-life applications where there are even slight temperature gradients or an assortment of materials which affect the process. It is effective against a broad range of organisms, but celluloid materials such as flooring, boxes, ceiling tiles, paperwork, etc. absorb the peroxide thereby scavenging it from other areas [JSPPTOH, 4/04]. Under standard conditions, vapor hydrogen peroxide is

a vapor, not a gas, which upon delivery to the room tends to transform back to its equilibrium state (liquid). Any cool surfaces or temperature gradients cause this to occur as condensation. This condensation on cooler surfaces caused by temperature gradients creates an uneven distribution of the decontaminating vapor. This creates areas with a greater amount of the decontaminating agent and areas with less or little, thereby not getting good distribution or more importantly good kill. Additionally vapor hydrogen peroxide has challenges in reaching difficult areas such as floor drains, HVAC grills, beneath furniture and components, the inside of cabinets, hinges, instruments and components. Vapor hydrogen peroxide was believed too problematic for the current application, particularly when interstitial spaces, ductwork and offices containing a lot of paper and celluloid materials were to be included within the decontamination.

Chlorine Dioxide
Chlorine dioxide (CD) is a greenish-yellow gas and is a single-electron-transfer oxidizing agent with a chlorine-like odor. Pure CD is an unstable gas and therefore is generated as needed. Although CD has "chlorine" in its name, its chemistry is radically different from that of chlorine. CD oxygenates products rather than chlorinating them and thus trihalomethane (THM's) formation does not occur (Long, B.W.). Therefore, unlike chlorine, CD does not produce environmentally undesirable organic compounds containing chlorine.

Chemical Formula ClO_2
Molecular Weight: 67.45 g/mole
Melting Point (°C): -59
Boiling Point (°C): +11
Density: 2.4 times that of air

Chlorine dioxide is a true gas at normal use temperatures and therefore is not susceptible to condensation issues and temperature gradients such as vapors. It has a yellowish-green color, which allows its concentration to be precisely monitored and controlled by a UV-VIS spectrophotometer. This allows tight process control from beginning to end of a decontamination cycle.

Chlorine dioxide (CD) has been recognized since the beginning of the century for its disinfecting properties. Gaseous CD has been shown to be more effective than liquid CD when applied in equal concentrations and times (Han,Y et al. 2001a). Chlorine dioxide in both gaseous and aqueous phase is a strong oxidizing agent and has about 2.5 times the oxidation capacity of chlorine (Bernarde at al., 1967). Additionally, CD gas has been approved for use as a sterilant/decontaminate by the United States Environmental Protection Agency (US EPA). Both gaseous and aqueous phase CD have been shown to be effective sanitizing agents that have broad and high biocidal effectiveness. Aqueous CD has been reported to effectively inactivate bacteria (Ridenour and Armbruster 1949, Benarde et al., 1965, Harakeh et al. 1988, and Foschino et al. 1998) including pathogens (Harakeh et al. 1985, Korich et al. 1990, Roberts et al. 1994), viruses (Noss et al. 1985, Chen et al. 1990), bacterial spores (Ridenour et al., 1949, Foegeding et al., 1986), and algae (White 1972).

Gaseous CD has been shown to be an effective disinfectant especially related to the use in the medical science areas. Rosenblatt et al. (1985, 1987 in US patent 4, 504, 442 and US patent 4, 681, 739) developed the use of gaseous CD to sterilize surfaces, especially the gas-impermeable surfaces of implements commonly employed in medical sciences, such as those made of porcelain, ceramics, metal, plastics, and glass. Jeng and Woodworth (1990) reported the sporicidal activity of CD gas under square-wave conditions within an experimental sterilizer used for medical implements. ClorDiSys Solutions, Inc. (Eylath et al 2003a and b) described the development of a process using CD gas for sterilization of an aseptic fill isolators and pharmaceutical process vessels.

Recently, gaseous CD has successfully been used to decontaminate B. anthracis contaminated areas of the Hart Senate Office Building and the Brentwood postal sorting facility in Washington, DC (Heilprin, 2001). Han et al. (2003a) also reported high efficacy of CD gas in reducing Bacillus spores on paper, plastic, epoxy-coated stainless steel, and wood surfaces. Additionally, numerous research has demonstrated that CD gas is highly effective (greater than five log reductions) in reducing foodborne pathogens (E. coli O157:H7, Listeria monocytogenes, and Salmonella) on fruits and vegetables surfaces (Han et al. 2000a and b, 2001a and b, 2002, Tsai et al. 2001, Du et al. 2002 and 2003, Sapers et al. 2003) and spoilage microorganisms on food-contact surfaces (Han et al. 1999a).

In addition, about 5% of large water treatment facilities (serving more than 100,000 people) in the United States use CD to treat drinking water. It is estimated that about 12 million people may be exposed in this way to CD. In communities that use CD to treat water for drinking uses, CD is permitted to be present at low levels in the tap water (ATSDR, 2002). It is also estimated that there are 743,015 pounds (337,026 kg) of CD released to the atmosphere from over 100 manufacturing, processing, and waste disposal facilities in 2000 (US Dept of H&HS, 2002).

Physical Site
The C. Mahlon Kline intensive care unit and neonatal intensive care unit comprises a nearly isolated building. The total volume of the target decontamination building was approximately 170,000 ft3. On the main floor were 15 patient units with a wooden slide door, appropriate facilities for providing food and waste disposal, two operating rooms, two laboratories, three restrooms, a pharmacy, several offices, and other smaller rooms. Additionally, there were two weighing stations built into the floor and an overhead hoist system to facilitate the transportation of patients and equipment. On the upper floor

were more offices, a restroom, kitchenette, and mechanical room housing the HVAC system. Office rooms on the upper floor remained furnished and contained files, computers, books, as well as other personal effects. Access to the upper floor was granted by a stairway and ladder. Two basement rooms were also connected to the main floor via two elevators. These rooms housed more mechanical and storage space. In addition to the occupied areas, the very dirty interstitial space above the basement and loft area were to be part of the decontamination process.

Site Preparation
Sealing the facility proved to be the most time-consuming, labor-intensive part of the project. This was because the building was not designed with the intent of filling the building with a decontaminating gas. All vents, external doors, dump holes, and openings along the exterior of the building were sealed using combinations of caulk, expanding foam, duct tape, and adhesive films. All plumbing was either capped or water was poured into floor and sink drains that had traps. All light fixture covers were removed. The two dump holes on the underside of the building were sealed. The building HVAC system was deactivated and the external vents sealed. External switches for these units were supplied to enable reenergizing them following the decontamination without having to enter the gas-filled space. Office windows that were capable of being opened were sealed, as were office air conditioning units. Access panels to interstitial spaces were opened within the main and loft floors.

Fans and blowers were distributed throughout the facility to ensure circulation of the CD gas. Several of these were placed at openings for the interstitial regions.

A blower was used to recirculate the gas through the main building exhaust and return units to force circulation throughout the HVAC system. Gauges to measure relative humidity and temperature were placed at several windows, allowing the internal conditions to be monitored during the decontamination. A total of ten chlorine dioxide gas generators and ten sample points (Table 1) were installed into the building. Generators were located as follows: six on the main floor, one for the stairwell, one for the basement, one for the loft area, and one for the HVAC system.

Once sealing the building was complete, a test of the building using isoamyl acetate (banana oil) was performed. Three pans with 200g isoamyl acetate and approximately 100ml water were placed upon hotplates within the building and the hot plates were energized to release the gas. Leaking sites were then sought by scenting for the isoamyl acetate along all external surfaces. Only one major leak at the wall adjoining the C. Mahlon Kline ICU/NICU to its adjacent building and several minor leaks along the roof were detected. Once these were repaired and retested the facility was ready for decontamination.

The Decontamination Event
The typical steps for chlorine dioxide (CD) decontamination are to raise the humidity to 65% to 70%, then hold this humidity (conditioning) for 30 minutes. Next inject the CD gas (charge) to the target concentration (typically 1 mg/L for rooms), then hold (exposure) for 60 - 120 minutes (typical for rooms) and finally aerate the gas by bringing in fresh air and exhausting up the stack. The actual decontamination steps used at the C. Mahlon Kline Intensive and Neonatal Care Units differed slightly. The humidity of the building was already high (70%) due to ambient humidity of 70%. So the conditioning step was not necessary since it had been occurring throughout the day. The charging step was first initiated and was set to bring the concentration up to 1mg/L of CD gas then hold for two hours. This time would yield a target exposure of 720 ppm - hours (1 mg/l 360 ppm). After one hour of charge the concentration was not building up as expected. See Table 2 for calculated times and actual times.

The lack of CD buildup to the expected concentration was most likely due to the large amount of materials in the facility reacting with the CD gas, materials such as papers, boxes, wood, concrete, dirt, and debris. Upon discovery of the charging issues, the cycle was then modified to continuing charging for the entire cycle time 10.5 hours and then initiate aeration. This reaction with materials was consistent with formaldehyde and vapor hydrogen peroxide. In fact, as stated above [JSPPTOH, 4/04], the reaction would have been much worse with the hydrogen peroxide. More importantly, since the CD concentrations were being monitored the cycle was modified. With formaldehyde and vapor hydrogen peroxide this would not have happened since the concentration is not monitored, so the cycle would not have been lengthened to get the desired results. The actual CD gas concentration can be seen in Table 3.

The target exposure as describe above was for 720 ppm-hours and this was expected to achieve a greater than six log reduction. The actual exposure concentration was approximately 400 ppm – hours.

Aeration was initiated after a 10.5 hour charge/exposure. The sealing materials from exhaust and intake fans on the roof were removed and the fans were energized. A CD odor was detected at ground level north of the building, but direct measurements in this area did not detect any CD. The detection level of the Draeger tubes used was 0.1ppm which is the eight hour (TWA) threshold as indicated by OSHA, 0.3 ppm being the short term exposure level (STEL). After 2.5 hours the levels of CD were no longer measurable and the building was safe to enter without personnel protective equipment. Note that the reduction of required aeration time from the anticipated six hours was consistent with the lower than planned concentration of CD at the onset of the aeration.

Results
A total of 100 biological indicators were placed throughout the building to validate the gaseous CD decontamination. Forty

(40) Geobacillus stearothermophilus spores (Spordex), twenty (20) Bacillus atrophaeus (Spordex); and forty 40 strips inoculated with the gram-negative bacteria Salmonella Newport. Among the locations of the biological indicators were four within the ceiling interstitial spaces and one within the HVAC return line.

Of the forty (40) biological indicators of G. stearothermophilus, thirty-two (32) of these produced no growth, seven (7) produced 1 CFU, and one (1) produced 2 CFU. There was no discernable pattern as to the location of the biological indicators that corresponded to nonzero CFU plates. None of the twenty (20) biological indicators of B. atrophaeus produced growth. Of the forty (40) test strips of Salmonella Newport thirty-eight (38) produced no growth (Table 4).

Conclusions

The decontamination process using chlorine dioxide gas was determined to be an unqualified success. Swab tests for Salmonella taken for two months post decontamination produced no positives. The University of Pennsylvania's C. Mahlon Kline Intensive and Neonatal Care Units was lifted from quarantine and declared fully operational.

Within three hours of the start of aeration, there were no chlorine dioxide odors. Furthermore, the preexisting "animal" odors were also absent. No physical residue was observed as would be if formaldehyde was used. Additionally there was no visible indication of material degradation on any of the metal containing equipment that had been left within the building including the scales, HVAC system, hoist system, etc. All mechanical and electrical items that had been present within the ICU/NICU during the decontamination, including typical office equipment such as computers and printers, continued to be functional.

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