Environmental Monitoring and Decontamination of Food Processing Facilities

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INTRODUCTION

In 1969, Elizabeth Kuebler-Ross published *On Death and Dying*, a book that described in detail the five stages of grief: Denial, Anger, Bargaining, Depression and Acceptance (DABDA). Although this is a troubling and disconcerting subject for most people, these stages often apply to many different situations, hardships or adversities. The five stages can even apply to the food industry, specifically to microbial contamination of the food processing environment.

Denial

In the first stage, management puts up a defense and convinces themselves that there is no issue or contamination. "We clean every day"…"Our cleaning and sanitation regimen is comprehensive and up to date"…"The lab must have messed up or contaminated our samples"…"The problem was not caused by us and did not come from our facility" are often heard. Or management can deny the existence of a problem by saying that the contamination is not on a food contact surface and therefore does not need to be addressed right away.

Anger

In the next stage, management expresses their anger over the situation loudly and/or ponders many questions. "Why, this is not supposed to happen to us!" "Who is responsible? They are going to lose their job over this." "Isn't our staff following our sanitation SOPs?" "We better find the source of the problem soon…or else."

Bargaining

This stage can occur before or after Anger. Management will start to think of ways to postpone dealing with the reality of microbial contamination while the lab results are being confirmed. "We will do anything to fix this problem"…"We just need more time"…"Can't do anything until we hear back from the lab," are common. They may also ask, "How did we get this problem?" "Do we maybe need to update our cleaning procedures?" "I bet it was the incoming raw materials. Did we check the raw ingredients?" "Should we do more product testing?" "If only we had done X then the contamination would not have happened."

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Depression

This stage sets in once the lab confirms sample contamination, and there is the realization that this could be a huge problem. This is especially so if the product itself is contaminated with pathogenic bacteria (such as *Salmonella* or *E. coli* O157:H7), that present a potential safety risk to their consumers. Management and staff start preparing for the worst. "This could be the end of our company." "Better start updating my resume and look for another job."

Acceptance

When the final stage sets in, management accepts that they have a contamination problem, and the focus changes to how to address the issue. "What do we need to do now?" "What solutions are there for cleaning and decontamination of the plant?" "How long do we need to shut down?" In the case of product contamination, other hard choices with huge implications must be made: "Do we ship or destroy the product?" "Should we initiate a recall?"

ENVIRONMENTAL MONITORING AND TESTING OF FOOD FACILITIES

Establishing and maintaining a comprehensive environmental monitoring program is critical to the food industry today, as it can serve as a system for early warning of potential contamination of the product. An effective and well-managed environmental monitoring program will include testing of microbiological risk areas in the plant, to find organisms before they get into the product, and will verify that all cleaning and sanitizing procedures are working effectively. When such a program is being created and revised, many questions must be answered: "Why test the environment?" "What organisms (bacteria and fungi) are of greatest concern?" "What microbiological limits are acceptable for our operation?" "Should we take samples from food contact surfaces and/or non-food contact surfaces?" "What locations should be sampled, and should a zone concept be adopted?" "Is air sampling necessary?"

The first step is to understand the enemy, or the microorganism(s) of concern.

- What is its primary habitat? Is it predominantly found in the intestines of warm-blooded animals (such as shiga toxin-producing *E. coli* in cattle), or is it a soil organism (such as *Listeria* spp. or *Clostridium botulinum*)? Sources can be very widespread for many microorganisms and can include "the great outdoors," food ingredients, the processing plant environment, pallets, drains, humans, and animals, including insects.
- What nutrients and conditions (water availability, oxygen, temperature, pH, etc.) are required for the organism to grow and survive?
- What steps are required to kill the organism (pasteurization, freezing, irradiation, acidic conditions)?
- Has the organism been implicated in foodborne illness outbreaks for the same or similar products?
- How many is too many? In the case of some bacteria, a high infectious dose is required for most individuals to exhibit symptoms (*Bacillus cereus* is about ~10^5 – 10^8 viable cells or spores), while for others, such as *E. coli* O157:H7, the infectious dose is very low (< 100 cells) *(11, 25)*. Initially, a great deal of testing may need to be done to thoroughly understand the plant environment and location of problematic niches. Once a baseline has been established, it will be easier to know what is "normal" for your operation after cleaning and sanitizing and it will also be easier to track trends.

Indicator organisms are commonly tested for in environmental monitoring programs, as they can reflect microbiological quality and show when conditions *may* permit growth of pathogenic organisms. These are typically done by enumeration tests in the laboratory and reported as CFU (colony forming units) or MPN (most probable number) per gram or mL. Examples of common indicator and spoilage organisms/laboratory tests include:

- Total Aerobic Plate Count (APC, SPC, TPC, HPC)
- Total Coliforms/Generic *Escherichia coli*
- *Enterobacter* (*Streptococcus* and *Enterococcus*)
- Fungi (yeast and mold)
- Lactic Acid Bacteria (acidophiles)
- Coagulase Positive *Staphylococcus aureus*
- *Bacillus cereus*
- *Listeria* genus/species

Depending on the food being produced and the consuming population, the concern may be for only indicator and spoilage organisms, or perhaps pathogenic bacteria also need to be included in the environmental monitoring program. Food pathogens continue to be a difficult challenge for all sectors of the food industry and pose a significant hazard to human health, causing approximately 48 million illnesses, 128,000 hospitalizations and 3,000 deaths each year *(33)*. The presence of these pathogenic

bacteria in the food processing plant can lead to costly product recalls, which can result in loss of revenue and customers, loss of prestige and brand reputation, bad publicity, legal fees, increased insurance premiums, and perhaps even closure. Here are some examples of pathogenic bacteria that are commonly tested for in the laboratory (and typically reported as presence/absence per 25 g, 100 g or 375 g):

- *Salmonella*
- *Listeria monocytogenes*
- *Escherichia coli* O157:H7/Shiga toxin-producing *E. coli* (STEC)/Enterohemorrhagic *E. coli* (EHEC)
- *Vibrio vulnificus*
- *Clostridium botulinum*/*C. perfringens*
- *Cronobacter* (*Enterobacter*) *sakazakii*
- *Campylobacter jejuni*

Another important step in setting up an environmental sampling plan is to completely know your food product, the target consumer group (children, the elderly, pregnant women and immunocompromised individuals are more susceptible to foodborne illness), and the environment in which the food is being produced. Some processing facility attributes to consider are the following:

- Type of processing (wet or dry)
- Plant cleaning and sanitation schedule
- Rotation of sanitizers
- Separation of production and storage areas
- The flow of product compared to worker traffic patterns
- Age and wear of equipment and facilities
- Presence of rust
- Floors, drains, roof and overhead concerns
- Standing water
- Air handling systems and dust
- Pest control and trash management

Finally, the individual or team responsible for establishing an environmental monitoring plan should think about all sampling locations and where to test. For food contact surfaces, some typical locations to consider are the following:

- Conveyors
- Tables
- Filling and packaging equipment
- Slicers and dicers
- Shredders and blenders
- Tanks
- Collators used to assemble and arrange produce
- Transport racks and other containers
- Spiral and blast freezers, or other solutions used to chill food
- Employee hands and gloves

For the above equipment, it is especially important to be on the lookout for poor or old designs, such as conveyor belting with fabric or worn seals for example, and also to not be afraid to disassemble to correctly take samples.

When it comes to non-food contact surfaces, it is all about going where no one has gone before, and knowing where all of the common "niches" are in your plant. Some examples of these hard-to-clean places that are known for harboring microorganisms include:

- Floors
- Carts
- Sewers and drains
- Drip pans
- Walls and windows
- Door seals
- Ceilings and other overhead structures (catwalks)
- Any structure with cracks in it
- Equipment framework
- Forklift tires and blades
- Cleaning and maintenance tools
- Trash barrels
- Insulation and condensation
- Sink areas and foot baths

Professional advice may be needed for help in designing a statistically sound environmental sampling and testing program for your specific food operation and for setting microbiological limits and specifications.

So, let's say a serious environmental contamination does occur in your facility, and you are getting samples that are positive for, say, *Salmonella* or *L. monocytogenes*. If this happens, then you need to temporarily close or limit access to the area, inspect the area closely (and disassemble all equipment), and collect more samples or re-swab to determine how widespread the contamination is. Then, it is time to **thoroughly** clean and sanitize everything. Quality and sanitation managers and supervisors should also be watching how their staff are cleaning, to make sure that they are following the procedures correctly. Before resuming operations, inspect and collect more samples again. When all tests have been negative for a set number of consecutive days, operations may resume.

If the problem continues to persist even after taking the above corrective actions, what happens then? Is it time to re-design the facility or buy new equipment? Or, are options available to completely eliminate all of the microorganisms?

INTRODUCTION TO ENVIRONMENTAL DECONTAMINATION OF FACILITIES

The United States Environmental Protection Agency (U.S. EPA) defines antimicrobial pesticides as substances or mixtures of substances used to destroy or suppress the growth of harmful microorganisms such as bacteria, viruses, or fungi on a variety of objects and surfaces.

Antimicrobial pesticides have two major uses:

- To disinfect, sanitize, reduce, or mitigate growth or development of microbiological organisms.
- To protect objects (e.g., floors and walls), industrial processes or systems, surfaces, water or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae or slime *(40)*.

The levels of kill as defined by the U.S. EPA *(40)* are:

- ***Sterilizers* (Sporicides):** Used to *destroy or eliminate all forms of microbial life including fungi, viruses, and all forms of bacteria and their spores.* Spores are considered to be the most difficult form of microorganism to destroy. Therefore, the EPA considers the term Sporicide to be synonymous with "Sterilizer."
- ***Disinfectants*:** Used on hard inanimate surfaces and objects to *destroy or irreversibly inactivate infectious* fungi and bacteria, but NOT necessarily their spores. Disinfectant products are divided into two major types: hospital and general use.
- ***Sanitizers:*** Used to *reduce*, but not necessarily eliminate, microorganisms from the inanimate environment to levels considered safe as determined by public health codes or regulations.
- ***Antiseptics and Germicides:*** Used to prevent infection and decay by inhibiting the growth of microorganisms. Because these products are used in or on living humans or animals, they are considered drugs and are thus approved and regulated by the Food and Drug Administration (FDA).

Once management has come to acceptance of the contamination, the next step is how to remove it. A few things are critical to achieving acceptable decontamination. The following items **must** be achieved in order for the decontamination to be acceptable:

- Good and complete distribution
- Good and total penetration
- Sufficient contact time
- Sufficient concentrations

Different types of decontamination methods

Several methods are available for decontamination. The most prevalent or most common method is spraying and wiping. In this method, the user sprays a liquid sanitizer/disinfectant/sterilant around the area to coat all surfaces. While this method is the most common, it is also the most fallible, because it is extremely difficult for the user to spray or wipe **all** surfaces within an area and keep them wet at the specified concentrations for the prescribed amount of time. For example, Luftman *(22)* described a *Salmonella* contaminated facility. That the users attempted to clean and decontaminate on two separate occasions; but were unsuccessful each time, because they could not reach all areas to fully decontaminate the facility. To eliminate the contamination at this facility, a gas-phase space decontamination was finally utilized. This method was successful because gassing complies with all four rules of decontamination.

Use of automatic foggers is another method, but it has the same limitation of not reaching all surfaces. In this method, an atomizer is utilized to create a fine mist (5 to 100 microns) and an attempt is made to coat all surfaces. This method is subject to gravity and room geometry, however, with odd shaped areas, equipment, and covered areas creating blind spots and shadow areas that the mist is unable to reach. When placing foggers within the space, it is critical to have a line of sight to all surfaces in order for the disinfectant to reach all surfaces. This is extremely difficult when equipment is in the room, as mists and fogs are unable to reach behind and below surfaces. Thus, the issues with foggers are distribution and penetration. It must be remembered that organisms are 0.5 to 2 microns in size, and can hide in niches too small for the 5+ micron mist to reach.

The next step in the liquid evolution is the ionized foggers. With this process, a 7.5% hydrogen peroxide solution is atomized (5 to 20 microns) and positively charged to allow the disinfectant to stick to surfaces. Although this helps with negatively charged surfaces, which most surfaces are, it creates problems with positively charged surfaces such as glass and aluminum. This method has limitations similar to those of conventional foggers, such as the difficulty

of reaching all surfaces and the need for a line of sight to satisfy all four rules of decontamination.

These limitations of reaching all surfaces bring fumigation into focus. For applications in which it is critical to reach all surfaces (such as a plant-wide contamination of pathogenic bacteria), fumigation is the process that achieves total coverage. Fumigation utilizes the excited state of a molecule (gas) to satisfy the four components of a successful decontamination. In this process, a gaseous agent is injected into the target chamber and gas laws control the work. In the fumigation arena, there are a few agents that can be used. There are the true gases (chlorine dioxide, formaldehyde and ozone) and the vapors (hydrogen peroxide dry process and hydrogen peroxide wet process). There are other gas processes (methyl bromide and ethylene oxide), but these will not be discussed, since each is associated with certain issues. Ethylene oxide is explosive *(26)* and methyl bromide is an ozone-depleting substance *(39)* that is being banned for most uses.

Formaldehyde gas is considered by many to be the fallback fumigation method. It has been used the longest *(28)* and has a long history of applications. In the past it had been approved by the United States Environmental Protection Agency (US-EPA) and is currently approved by NSF for biological safety cabinet decontamination *(27)*. Its use has been diminishing all over the world because of concerns over carcinogenicity *(16)* and the residues it creates, either paraformaldehyde or methenamine *(20)*.

Vapor phase hydrogen peroxide (VPHP) was the next method to be developed *(29)*. It has benefits over formaldehyde, such as that it does not leave residues and is considered by most to be non-carcinogenic; IARC, NTP, and OSHA do not list hydrogen peroxide as a carcinogen. Hydrogen peroxide is listed as an A3 animal carcinogen by the ACGIH. The vapor phase is generated by boiling or vaporizing a solution of hydrogen peroxide, which is typically 35% hydrogen peroxide and 65% water. This vapor phase is then injected into the room or target chamber. There are two views toward using VPHP. One method uses a "dry" process, in which the relative humidity (RH) is lowered to maximize the amount of vapor in the air and the vapor is maintained in the dry state to maximize distribution of the vapor. The other method, a "wet" process, allows the vapor to condense on surfaces, as the RH is not lowered prior to injection. This promotion of condensation limits the distribution of the vapor as gravity causes droplets to fall before they can reach surfaces farther away. To maximize distribution, the VPHP generator is typically placed inside the area during the "wet" process. Either process will have varying amounts of condensation since VPHP is not a true gas at room temperatures (hydrogen peroxide's boiling point is 109°C) and RH levels can typically exceed 90% *(31)*.

When VPHP condenses, its concentration increases to a maximum concentration of 78% hydrogen peroxide *(15)*. Although the increased concentration is beneficial for decontamination, this concentrated oxidizer can cause surface damage to painted surfaces *(15, 24)* and epoxy surfaces *(10, 32)*. Another issue with VPHP is it has poor distribution *(14, 35)* and low penetration ability into 5 mm gaps *(37)* and small tubing and openings *(7)*. One other concern with VPHP is it's cycle development. Not all rooms or target areas are the same. Even if room volume is the same, if the room layout has changed, the cycle may differ. Fans must be placed to hit every corner or hard-to reach surface in the room *(32)*.

The latest, or next generation, fumigant method uses chlorine dioxide (CD) gas. CD gas is a true gas at room temperature and because of that shares the same advantages that formaldehyde does in distribution and penetration, being able to contact all surface, including cracks and crevices. It has been used in studies *(13, 17)*, isolators *(5, 8)*, processing vessels *(9)*, juice tanks *(12)*, HEPA housings with small tubing *(7)*, BSCs *(23, 27)*, rooms *(19, 32)*, and large facilities *(4, 22)*. It is a gas at room temperature (boiling point 11°C) like formaldehyde, and is not considered to be carcinogenic by IARC, NTP, OSHA or ACGIH. Like VPHP, it does not leave a residue, but unlike vapor phase hydrogen peroxide CD, it is a true gas. So CD gas has the benefits of formaldehyde without the carcinogenic and residue drawbacks, and has the benefits of VPHP (no residues and fast cycle times) without the poor vapor distribution and penetration associated with VPHP.

Preparing the facility for fumigation

The steps necessary for fumigating a target space are similar with any of the fumigants. For this article, the discussion will focus on chlorine dioxide gas fumigation. Before fumigation or decontamination can take place, the area must be **thoroughly** cleaned. This cleaning step removes all bioburden and food particles and allows the decontamination agent to get to the organism(s) of concern. This step usually takes the form of deep scrubbing, sweeping, vacuuming and washing, concluding with the proper sanitizing agent. When the cleaning step is completed (and depending on which fumigation agent is to be used), the space may need to be completely dry. VPHP does not penetrate water, so all surfaces must be dry. Chlorine dioxide, on the other hand, does absorb or diffuse into water and maintains its efficacy, so all surfaces do not need to be dry. All decontamination agents are affected by organic loads, which is why cleaning must take place. Chlorine dioxide has shown promising results with organic loads, such that studies at Public Health Agency of Canada show kill with organic soiled loads *(18)*, wood, carpet and ceiling

FIGURE 1. HVAC Main Supply

tiles *(30)*, under mouse cage bedding *(36)* and HEPA filters loaded with soil *(23)* which raises the question, How clean is clean?

As with any fumigant, the space must be properly sealed to contain it. This means sealing all points of entry or exit, such as personnel doors, loading dock doors, elevators, windows, and HVAC supply and exhaust. The facility or building layout is an important drawing for this, as it identifies all the access points that will require sealing. It also gives the volumes requiring decontamination, which is necessary to determine the amount of generators necessary to decontaminate the space. The other drawing that may be necessary is the HVAC drawing. This identifies which area is part of which HVAC system, as some facilities have multiple HVACs servicing several different areas. Most of the time, the on-site HVAC technician is the most important facility person to assist in the job. This person is **usually** aware of which HVAC services which area, and also knows of any modifications that have been made. In most circumstances, the HVAC is utilized to exhaust the gas after exposure (federal, state and local laws must be followed). The HVAC supply and exhaust needs to be sealed, and the HVAC technician can assist in this. Sometimes it is easier to seal on the outside of the building, and other times it is easier to seal inside the HVAC unit itself (Fig. 1).

Fumigation can be costly, so the gassing areas should be narrowed down to only the necessary areas. Looking at the layout of the facility with the contaminated areas highlighted helps to identify the important areas and to determine whether areas are separate or can be joined together to form one big target volume. The larger the volume, the greater the cost. Additionally, the larger the area the more effective the entire process will be, because the contamination is reduced or eliminated over a wider area.

Once the HVAC is sealed, the gassing generators must be set up with gas injection tubing run from outside the decontamination area into the target decontamination space. Generators are normally placed outside the area for safety reasons. Typically, ¼" (6.35 mm) plastic polyethylene (PE) tubing is used. Once the gas injection tubing is run, the sample tubing (PE) is placed in the locations. This sample tubing is typically placed in the back of rooms away from the injection tubing. The injection tubing is typically placed in the center of the room or in hallways that are central to the whole facility. After the tubing is placed; the distribution fans are placed; and these are typically one 12" (30.48 cm) fan per room for sizes up to 2,000 ft^3 to 3,000 ft^3 (56.63 m^3 to 84.95 m^3. For larger rooms (10,000 ft^3 to 20,000 ft^3 (283.17 m^3 to 566.34 m^3), larger-sized fans are used, also typically one fan per room. A similar number of fans is required for formaldehyde. For gas applications, the fans create turbulence to produce a uniform gas mixture quickly. For vapor applications, more fans are required than with gas *(32)*. The RH must also be raised to a minimum of 65% after fan placement. Moisture is **critical** for all spore log reductions, no matter what agent is used (formaldehyde, CD gas, or VPHP) *(2, 41, 42)*. With the dry vapor process, the air is typically dried prior to VPHP injection. During the VPHP injection (wet or dry process), 65% water is also injected and increases the RH to over 90% *(31)*. The moisture necessary for spore log reduction is supplied with the VPHP process.

The last step to be performed before starting the gassing is to strategically place the biological indicators (BIs) which are used as a quality check and to test the efficacy of the process. For chlorine dioxide gas, *Bacillus atrophaeus* (ATCC 9372) is typically used, because this organism is the most resistant to chlorine dioxide gas. This resistance has been documented by Jeng, who showed that *B. subtilis* var. *niger* (now renamed *B. atrophaeus*) was more resistant *(17)*. When validating the use of CD gas for BSCs, Luftman chose *B. atrophaeus* over *Geobacillus stearothermophilus (21)*. Czarneski also demonstrated a greater resistance of *B. atrophaeus* to CD gas *(6)*. The number of BIs and placement of BIs can be a challenge. Some people try to put a certain

number per cubic foot volume, but this can be challenging, since some facilities can be 500,000+ cubic feet (14,158). If the number of rooms is small, then the number of BIs is small, and if the number of rooms is large, then the number of BIs is large. However, what if the number of rooms is small, but the volume is large? The common sense approach for the number of BIs and location of BIs would be to place them in areas that are of greatest concern.

The Fumigation process

Once all of the equipment is in position and BIs are placed, the decontaminating cycle can begin. It is begun by raising the relative humidity to a minimum 65% RH for 30 minutes, after which the gassing of the facility can commence. CD gas is then introduced to reach a concentration of approximately 1 mg/L (362 ppm) and held at that level for 2 hours. The CD gas concentration is easily monitored with a validated *(34)* UV-VIS photometric device (ClorDiSys EMS module) that displays the real-time concentration in mg/L. The concentration-exposure time (CT) of 1 mg/L for 2 hours equates to 720 ppm-h (362 ppm * 2 h = 722 ppm-h). By using the CT exposure, accumulation of ppm-h starts during the charging phase when the gas concentration is increasing. This allows for shorter cycle times, since the ppm-h are accumulating before the target concentration is reached. Czarneski summarized the results in various publications which validate CT or ppm-h. So when the CT time is 720 ppm-h or greater, the exposure time is done *(6)*.

The next step is aeration, in which the gas is removed by energizing the HVAC supply and exhaust. The exhaust removes the gas and the HVAC supply adds clean air to the target chamber. When the concentration read by the EMS module reaches 0, the cycle is complete. Before the chamber is entered, it must be checked with a low level safety sensor to ensure that the gas is down to safe levels for chlorine dioxide gas (0.1 ppm OSHA PEL [personnel exposure level time weighted average]). Once the chamber is fully aerated, no other steps are necessary and it can be used again. The fumigation equipment is then removed, and BIs are removed and incubated to determine the results. Personnel entering the facility may or may not be gowned as per the facility's standard procedures .

CONCLUSIONS

Microbial contamination of food facilities continues to be a difficult challenge for the industry and can present a significant health hazard to human safety when disease-causing microorganisms get into the final food product. It is a very troubling and stressful issue for all parties involved, as the similarity of the response to contamination and the five stages of grief demonstrated in the opening paragraphs of this paper. This is especially true if a recall has to be initiated, if consumers become ill, if the media get involved, and if there is a potential for closure. Many pieces have to come together in the big food safety puzzle, and food processors who have a comprehensive environmental monitoring program constitute one piece of the puzzle. A well-maintained program will include microbiological testing of the risk areas in the plant, to locate the organisms before they get into the product, and to verify that cleaning and sanitizing procedures are effective. Professional advice may be needed, though, to help establish and manage sampling and testing programs for your specific food operation.

If a serious and widespread environmental contamination does occur in the facility, then fumigation may be your answer; as it provides a decontamination method to eliminate pathogens **completely**. There are many ways to decontaminate spaces. Regardless of which method is chosen, the agent or technology must achieve all four aforementioned rules of decontamination. The agent must have complete distribution, good penetration, and sufficient contact time at the required concentration. The most common method, spraying and wiping, happens to be the most deficient, as it relies on the staff to spray and wipe ALL surfaces, and use ladders to clean hard-to-reach areas or tall equipment. This is the biggest challenge with manual methods, the ability to reach everywhere, which is why foggers are used. It is basically the same approach, but the user is removed from the process. So, while removing the person from the process is good, it still is very challenging for fogs or mists to reach all nooks and crannies, because they rely upon line of site and are subject to gravity, being composed of small liquid droplets. Both fogger methods, along with manual spraying and wiping, have the drawback of leaving residue. Since liquids are sprayed on surfaces, these liquids dry and form deposits.

Because manual methods and foggers have limited abilities, fumigation is the preferred method, as the outcome is not dependent on the user. It is important to remember that not all fumigation methods are equal, though. Formaldehyde, considered by many to be the grandfather or the tried and true, is inexpensive, easy and effective; it has two significant drawbacks, though: it is a carcinogen and it leaves residues. VPHP, used in the method developed after formaldehyde gas, does not have the same drawbacks of residues or being a carcinogen and is more effective than spraying and wiping or fogging, but it presents problems because it is a vapor and not a true gas at room temperatures and because of this has difficulty penetrating or diffusing. Niches in food

processing plants can harbor pathogenic microorganisms, which is why it is critical for the decontaminating agent to reach these areas. Besides penetration issues, some consider VPHP to be a carcinogen, and it has also shown compatibility issues with some paints and epoxy floors when condensation occurs.

Chlorine dioxide gas, the latest fumigant, has the benefits of formaldehyde, in that it is a true gas at room temperatures, but it is not considered by any organization to be carcinogenic. Because it is a true gas, it does not have the distribution or penetration limitations of VPHP, and it has demonstrated excellent decontamination abilities *(39)*, does not leave any residues behind, and has good material compatibility. While all methods can be effective it is only chlorine dioxide that achieves the required and necessary coverage without the drawbacks of other methods. This is the reason it was used in most of the spaces (Hart Senate Office Building, Brentwood P& DC Trenton P&DC and AMI Bldg) contaminated with anthrax in 2001.

REFERENCES

1. Ackland, N. R., M. R. Hinton, and K. R. Denmeade. 1980. Controlled formaldehyde fumigation system. *Appl. Environ. Microbiol.* 39:(3):480–487.
2. Agalloco, J., P. Carleton, and J. Frederick. 2008. Validation of pharmaceutical processes. (3rd ed.). Informa Healthcare USA Inc. New York, NY.
3. Czarneski, M. A. 2007. Selecting the right chemical agent for decontamination of rooms and chambers. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc*. 2:85–92.
4. Czarneski, M. A. 2009. Microbial decontamination of a 65-room new pharmaceutical research facility. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc*. 2:81–88.
5. Czarneski, M. A., and P. Lorcheim. 2005. Isolator decontamination using chlorine dioxide gas. *Pharma. Tech.* 4:124–133.
6. Czarneski, M. A., and P. Lorcheim. 2008. Validation of chlorine dioxide sterilization, p. 281–287. *In* J. Agalloco and F. Carleton (eds.), Validation of pharmaceutical processes (3rd ed.). Informa Healthcare, USA. New York, NY.
7. Devine, S., K. Woolard, and A. Mahler. 2009. Challenges encountered in decontamination of small spaces and tubes, 52nd Annual Biological Safety Conference, October 15–21, 2009, Miami, FL.
8. Eylath, A., D. Wilson, D. Thatcher, and A. Pankau. 2003. Successful sterilization using chlorine dioxide gas: Part one—Sanitizing an aseptic fill isolator. *BioProcess Intl*. 7:52–56.
9. Eylath, A. S., E. R. Madhogarhia, P. Lorcheim, and M. A. Czarneski. 2003b. Successful sterilization using chlorine dioxide gas: Part two—Cleaning process vessels. *Bio-Process Intl.* 8:54–56.
10. Feldman, L. A., and H. K. Hui, Henry K. 1997. Compatibility of medical devices and materials with low-temperature hydrogen peroxide gas plasma. Available at: http://epotek.com/SSCDocs whitepapers/Tech%20Paper%2059.pdf. Accessed 31, July, 2012.
11. Granum, P. E. 2007. *Bacillus cereus*. *In* M. P. Doyle and L. R. Beuchat (eds.), Food microbiology: Fundamentals and frontiers (3rd ed.). Washington D.C.: ASM Press.
12. Han, Y., A. M. Guentert, R. S. Smith, R. H. Linton, and P. E. Nelson. 1999. Efficacy of chlorine dioxide gas as a sanitizer for tanks used for aseptic juice storage. *Food Microbiol*. 1999, 16:53–61.
13. Han, Y., D. M. Sherman, R. H. Linton, S. S. Nielsen, and P. E. Nelson. 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157: H7 to green pepper surfaces. *Food Microbiol*. 5:521–533.
14. Herd, M. 2005. 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, and B. Martin (eds). Washington, D.C.: Office of Research and Development, U.S. Environmental Protection Agency. Contract No. EP-C-04-056.
15. Hultman, C., A. Hill, and G. McDonnell. 2007. The physical chemistry of decontamination with gaseous hydrogen peroxide. *Pharma. Eng.* 1:22–32.
16. International Agency for Research on Cancer (IARC). 2004. Available at: http://www.iarc.fr/en/media-centre/pr/2004/pr153.html. Accessed 7-31-2012.
17. Jeng, D. K., and A. G. Wooworth. 1990. Chlorine dioxide gas sterilization undersquare-wave conditions. *Appl. Environ. Microbiol.* 56:514–519.
18. Krishnan, J., G. Fey, J. Berry, and S. Wagener. 2006. Biodecontamination studies using vaporous hydrogen peroxide (VHP) and gaseous chlorine dioxide (GCD). American Biological Safety Association Annual Biosafety Conference, October 15–18, 2006, Boston, MA.
19. Leo, F., P. Poisson, C. S. Sinclair, and A. Tallentire. 2005. Design, development, and qualification of a microbiological challenge facility to assess the effectiveness of BFS aseptic processing. *PDA J. of Pharma. Science and Tech.* 1:33–48.
20. Luftman, H. S. 2005. Neutralization of formaldehyde gas by ammonium bicarbonate and ammonium carbonate. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc.* 2:101–106.
21. Luftman, H. S., and M. A. Regits. 2008. *B. atrophaeus* and *G. stearothermophilus* biological indicators for chlorine dioxide gas decontamination. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc.* 3:143–157.
22. Luftman, H. S., M. A. Regits, P. Lorcheim, M. A. Czarneski, T. Boyle, and H. Aceto. et al.

2006. Chlorine dioxide gas decontamination of large animal hospital intensive and neonatal care units. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc.* 3:144–154.
23. Luftman, H. S., M. A. Regits, P. Lorcheim, K. Lorcheim, and D. Paznek. 2008. Validation study for the use of chlorine dioxide gas as a decontaminant for biological safety cabinets. *Appl. Biosafety: J. of the Amer. Biol. Safety Assoc.* 4:199–212.
24. Malmborg, A., M. Wingren, P. Bonfield, and G. McDonnell. 2001. VHP takes its place in room decontamination. *CleanRooms*, 11.
25. Meng, J., M. P. Doyle, T. Zhao, and S. Zhao. 2007. Enterohemorrhagic *Escherichia coli*. *In* M. P. Doyle and L. R. Beuchat (eds.). Food Microbiology: Fundamentals and frontiers (3rd ed.). Washington D.C.
26. National Institute for Occupational Safety and Health (NIOSH) and Centers for Disease Control and Prevention (CDC). 1994. International chemical safety cards, ethylene oxide. Available at: http://www.cdc.gov/niosh/ipcsneng/neng0155.html. Accessed 7-31-2012.
27. *NSF International Standard / American National Standard*. NSF/ANSI 49 – 2008. Biosafety Cabinetry: Design, Construction, Performance, and Field Certification. Prepared by The NSF Joint Committee on Biosafety Cabinetry. Published by *NSF International*, P.O. Box 130140, Ann Arbor, Michigan 48113–0140, USA.
28. Nordgren, G. 1939. Investigations on the sterilization efficacy of gaseous formaldehyde. *J. Amer. Med. Assoc.*19:1759–1760.
29. Moore, F. C., and L. R. Perkinson. 1979. Hydrogen peroxide vapor sterilization method. U. S. Patent 4,169,123. Sept. 25.
30. Rastogi, V. K., L. Wallace, L. S. Smith, S. P. Ryan, B. Shawn and M. Blair. 2009. Quantitative method to determine sporicidal decontamination of building surfaces by gaseous fumigants, and issues related to laboratory-scale studies. *Appl. Environ. Microbiol.* 11:3688–3694.
31. Rogers, J. V., Y. W. Choi, and W. R. Richter. 2011. Effects of drying and exposure to vaporous hydrogen peroxide on the inactivation of highly pathogenic avian influenza (H5N1) on non-porous surfaces. *Appl. Biosafety.* 1:4–8.
32. Sawyer, M. 2010. Biosecurity Research Institute Kansas State University. Got Gas? Chlorine Dioxide or Vaporized Hydrogen Peroxide: Which one is right for you?, MABION The Midwest Area Biosafety Network Symposium, Ames Iowa.
33. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis.* 1:7–15.
34. Shah, S., T. Sickler, L. Smith, L. Wallace, and V. Rastogi. 2005. Validation of photometric measurement of chlorine dioxide gas. Conference on Chemical and Biological Defense Research. November 14–16, Timonium, MD.
35. Shearrer, S. 2006. Comparison of formaldehyde vs. VHP decontamination within operational BSL-4 laboratory at Southwest Foundation for Biomedical Research, San Antonio, Texas. 49th Annual Biological Safety Conference Program. October 15–18, 2006. Boston, MA.
36. Sidelsky, M. 2008. Design Decisions for Decontamination, Presented at Turnkey 2008, Baltimore MD, April 18, 2008.
37. Steris Corp. 2000. Industry Review: Room decontamination with hydrogen peroxide vapor, Publication ID #M1941EN. 2002–09 Rev. C, 2000.
38. U.S. Environmental Protection Agency (EPA). 1993. Environmental Protection Agency, 40 CFR Part 82, Protection of stratospheric ozone, 58(236), 65018 (Rule) 8006.
39. U.S. Environmental Protection Agency (EPA) EPA/600/R-05/036. 2005. Building decontamination alternatives, Section 5.1.4.2 Experience with field fumigation of buildings. Washington, D.C.: U.S. Environmental Protection Agency National Homeland Security Research Center Office of Research and Development. Prepared by Science Applications International Corporation.
40. U.S. Environmental Protection Agency (EPA). 2011. What are antimicrobial pesticides? Available at: www.epa.gov/oppad001/ad_info.htm.
41. Westphal, A. J., P. B. Price, T. J. Leighton, and K. E. Wheeler. 2003. Kinetics of size changes of individual *Bacillus thuringiensis* spores in response to changes in relative humidity. *Proceedings of the National Academy of Sciences* 6:3461–3466.
42. Whitney, E. A. S., Beatty, M. E., Taylor, Jr., T. H., Weyant, R., Sobel, J., Arduino, M. J., et al. 2003. Inactivation of *Bacillus anthracis* spores. *Emerg. Infect. Dis*. 6:623–627.