A Discussion of Biological Safety Cabinet Decontamination Methods: Formaldehyde, Chlorine Dioxide, and Vapor Phase Hydrogen Peroxide

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Abstract

This article discusses the three primary methods utilized for the decontamination of Class II biological safety cabinets (BSCs). Primary methods for BSC decontamination are formaldehyde gas, vapor phase hydrogen peroxide, and chlorine dioxide gas. Formaldehyde is inexpensive and easy to use; however, it is a possible carcinogen and leaves residues. Both vapor phase hydrogen peroxide and chlorine dioxide gas have the benefit of leaving no residues, but each is an oxidant which may affect materials. The costs for vapor phase hydrogen peroxide are high due to the required equipment; the costs for chlorine dioxide gas can range from low to high depending upon the equipment selected. The cost for formaldehyde is recognized by all as inexpensive in consumables and equipment.

Introduction/Background

Biological safety cabinets are used to protect the user from harmful agents and provide product and environmental protection. Airflow, within the cabinet and outside of the cabinet, is drawn into the front grille of the cabinet, thereby providing user protection. The filtered downward laminar flow onto the work surface provides product protection, and the exhaust through a HEPA filter provides environmental protection. All three types of protection are from biological agents which a laboratory may use. According to NSF International (formerly the National Sanitation Foundation) (NSF/ANSI 49 Class II [laminar flow] Biosafety Cabinetry), BSC decontamination is required when any maintenance work, filter changes, or performance tests require access to any part of the cabinet that may be contaminated. BSCs should also be decontaminated prior to decommissioning, salvage, or moving. If a BSC has not been decontaminated, pathogenic agents used within the BSC may be released and potentially expose the person performing the service or contaminate the environment.

NSF International is a not-for-profit, non-governmental organization providing educational information, standards, and product certification. The NSF Biosafety Cabinet program was started over 25 years ago at the behest of Centers for Disease Control and Prevention (CDC), National Institutes of Health (NIH), and the National Cancer Institute (NCI). NSF International has developed standards for Class II laminar flow biological safety cabinets (NSF/ANSI Standard 49). The standards define the basic design, construction, and performance that the BSC must adhere to in order to be certified by NSF.

NSF International provides guidance for the decontamination of BSCs. The guidance is provided by pre-validating chlorine dioxide gas and providing procedures for formaldehyde gas. This allows the user to follow cycles previously validated and to have confidence, if the steps are followed, that the decontamination will be successful. These procedures allow the user to service the BSC immediately after decontamination without having to place biological indicators (BIs) and wait the 3-7 days for incubation of the exposed BIs. Servicing a contaminated cabinet presents risks from the biohazards used in the cabinet. For example, opening a BSC in which tuberculosis (TB) work was performed would put the service person at risk of acquiring a *Mycobacterium tuberculosis* infection. However, the risks of working with sterilants or decontaminating agents that kill these organisms can also be hazardous. For example, the OSHA permissible exposure level (PEL) for hydrogen peroxide is 1.0 ppm and the levels inside the cabinet or chamber can exceed 1,000 ppm (Mispagel, 2009). For chlorine dioxide the levels can exceed 1,800 ppm (0.1 ppm PEL) and formaldehyde can exceed 8,000 ppm (0.75 ppm PEL). In all cases the exposure concentrations are much greater that the PELs, so leaks with any method can become an issue. The BSC service personnel must be aware that the decontaminating agents are killing organisms and that people are also at risk. Any agent that kills organisms is hazardous to people. These points are made to remind the user that organisms used inside the BSC provide a health hazard and the decontamination agent to mitigate the risk of the organism is also dangerous.

There are three primary methods for decontaminating BSCs: formaldehyde gas, vapor phase hydrogen peroxide, and chlorine dioxide gas. Formaldehyde has been used the longest, having first been used scientifically in 1939 when it was tested on 70 different bacteria types (Nordgren, 1939). NSF recommended it as early as 1992 (NSF Standard 49, 1992). Vapor phase hydrogen peroxide was developed in the 1970s (Moore, 1979) for sterilization and decontamination and is currently not recommended by NSF for BSC decontamination. Chlorine dioxide gas was developed in the 1980s for decontamination and sterilization (Rosenblatt, 1987) and was recommended by NSF in 2008 (NSF Standard 49, 2008).

When discussing decontamination and choosing an agent, the user must contemplate various aspects. For example, the user must not look solely at one feature and make a choice based upon that, such as which has the fastest cycle. Aspects worth considering are (Table 1):

1. What is the OSHA permissible exposure level (PEL) or threshold limit value (TLV) of the decontaminating agent?
2. Does the agent have an odor threshold at or below the OSHA PEL or TLV? If the agent has an odor, it can provide a self-alerting factor.
3. How long is the cycle time? This is the time a deadly agent is present. Cycle time should be minimized to reduce the possibility of accidentally creating a leak. The longer the cycle time the higher the possibility of accidents happening.
4. Is the agent a carcinogen or not?
5. What are the typical concentrations in the BSC?
6. What are the penetration and distribution abilities? Can it penetrate HEPAs?
7. Does the agent penetrate water? Oil? Grease?
8. Also, if an accident does occur, how quickly can the chamber be aerated? How quickly can the environment be made safe if a severe accident or leakage occurs?
9. Does the agent leave a residue?
10. Is the agent NSF recommended?
11. Is the agent approved by the U.S. Environmental Protection Agency (EPA)? EPA regulates the sale and use of pesticides and antimicrobial pesticides under the statutory authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and any agent used must be registered with the EPA.

To ensure safety, the user must properly seal the BSC to contain the decontaminating agent and have contingency plans in the event of small or large leakage. In the event of small leakage, the user can use low-level sensors (Analytical Technology, Inc. (Collegeville, PA), Interscan Corporation (Chatsworth, CA), Draeger USA (Pittsburgh, PA) to locate the leak and correct any issues. If the leak approaches unsafe levels or exceeds the OSHA PEL, the user must start the process of either neutralizing or aerating the decontamination agent. This step removes the decontamination agent to make the environment safe for personnel.

For space decontamination of the BSC, NSF-recommended methods are depolymerized paraformaldehyde and chlorine dioxide gas. Each of these methods has undergone various studies to determine the effective concentrations and exposure times to ensure a proper decontamination. NSF has provided procedures that when followed provide a means to decontaminate a BSC. NSF also allows the user to use other decontamination agents, such as vapor phase hydrogen peroxide (VPHP). Prior to using any other methods, such as VPHP, the cycle parameters must be developed and validated for each cabinet model and size.

For any decontamination to be successful, the user must first identify the requirements or demands on the agent used. The agent used must attain three critical parameters (Czarneski, 2007):

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

When appraising any agent's effectiveness, it must be evaluated with respect to these parameters.

Formaldehyde Gas

Formaldehyde is the most commonly used space-decontaminating agent because it has been used for space decontamination of BSCs and rooms the longest (Nordgren, 1939). It is used primarily because it is a true

Table 1
Summary

		CD	VPHP	Formaldehyde
1.	OSHA 8 hr TWA (time weighted average)	0.1 ppm	1.0 ppm	0.75 ppm
2.	Odor Detection	Yes	No	Yes
3.	Cycle Time (Risk of Exposure)	3-4 hours	4-7 hours	9-15 hours
4.	Carcinogen	NO	NO[1]	YES
5.	Typical Concentrations	1800 ppm	1000 ppm	8000-10000 ppm
6.	Penetration & Distribution	Yes (gas)	No (Vapor)	Yes (gas)
7.	Penetrate Water	Yes	No	Yes
	Penetrate Oil	No	No	No
	Penetrate Grease	No	No	No
8.	Emergency Aeration Time	5-30 min	1-6 hours	1 hour + cleanup
9.	Residues	None	None	Yes
10.	NSF approvals	Yes	No	Yes
11.	U.S. EPA approvals	Yes	Yes	No

[1]IARC, NTP, and OSHA do not list hydrogen peroxide as a carcinogen. ACGIH lists hydrogen peroxide as an A3 animal carcinogen.

gas at room temperatures and has good penetration abilities. To generate the gas, simple electric hot plates or commercially available automatic generators (Certek Nextek Model #1414RH* Formaldehyde Generator/ Neutralizer [Certek, Durham, NC]) can be used.

One issue with formaldehyde is its residue. This residue (either paraformaldehyde or methenamine [Luftman, 2005]) must be cleaned from all work surfaces. Residual formaldehyde due to off-gassing from the paraformaldehyde is a concern because of its toxic and irritating properties and potential for adverse effect on the research being conducted in the BSC. Cleaning the residue is usually done by wiping down all surfaces within the BSC, which is difficult to accomplish in the plenums, blower modules, below the work surface, etc., with an ammonia-based solution. Note: formaldehyde is considered a probable human carcinogen by the U.S. EPA (U.S. EPA, 1999) and is classified as a carcinogen to humans by the International Agency for Research on Cancer (IARC, 2004).

The following steps for formaldehyde decontamination are paraphrased from NSF 49 Annex G Part 7 and are applied to all class II (A1, A2, B1, and B2) cabinets. To perform a formaldehyde decontamination, the user weighs 0.30 g/ft^3 (11 g/m^3) of volume within the BSC. All areas or spaces the gas needs to penetrate must be included in the volume calculations. Once the volume of the cabinet is determined, the cabinet must be sealed to contain the sterilant gas. If the BSC is ducted, the damper must be closed and air tight. This will eliminate the loss of gas into the exhaust system. Sometimes it may be necessary to decontaminate the exhaust system, so this additional volume must be accounted for. To ensure proper decontamination with formaldehyde gas, the cabinet must first be conditioned with relative humidity (RH). The proper RH levels for formaldehyde are 60%-85% (Agalloco, 2008; Spiner & Hoffman, 1971; Westphal 2003; Whitney, 2003). This moisture or conditioning causes swelling of spores; which increases the diameter of channels through which decontaminating agents can pass (Westphal, 2003). Once the RH is at the proper level, the gas can be introduced. This is typically done by heating up the proper amount of paraformaldehyde which releases the formaldehyde gas. Once this has occurred, the BSC must be exposed to the concentrations for a minimum of 6 hours and preferably 12 hours. During the heating process, the BSC blower must be energized for 10-15 seconds when 25%, 50%, 75%, AND 100% of the paraformaldehyde has depolymerized. Concentrations within the BSC can reach 8,000-10,000 ppm (Luftman, 2005). The blower activation or "bumping" allows the gas to penetrate to all areas of the BSC including above the outlet HEPA filter (clean side). If the blower is inactive or not working, a recirculation system must be set up to get the gas above the exhaust HEPA filter. Once the exposure time has elapsed, the gas needs to be vented, scrubbed, or neutralized. To neutralize the amount of ammonium bicarbonate necessary is determined by multiplying the amount of paraformaldehyde originally used by 110%. When the formaldehyde exposure is completed, the ammonium bicarbonate is heated up to release the ammonium vapor which neutralizes the formaldehyde gas. A minimum of 1 hour must elapse for neutralization. The blower must be bumped during the process to ensure the ammonium vapor reaches all areas. The neutralization process creates a residue consisting of repolymerized formaldehyde (paraformaldehyde dependent upon conditions) and the neutralization product (methenamine) (Luftman, 2005). If the cabinet is ducted, the damper can be opened to exhaust the gas. (Federal, state and local laws must be followed.) The formaldehyde gas can also be scrubbed with a carbon-based scrubbing system to remove or scrub the gas from the air stream. Once the formaldehyde is removed, the decontamination is completed and the cabinet service can begin.

Chlorine Dioxide Gas

Like formaldehyde, chlorine dioxide (CD) is a true gas. It has been used for years in chambers such as isolators (Czarneski, 2005; Eylath, 2003a), processing vessels (Eylath, 2003b), rooms (Leo, 2005), and large facilities (Czarneski, 2009). Benefits of CD gas are that it does not leave a residue compared to formaldehyde and is considered to be non-carcinogenic by IARC, the National Toxicology Program (NTP), OSHA, and the American Conference of Industrial Hygienists (ACGIH). Since CD is a gas at room temperatures, it does not condense on surfaces. Furthermore, it is not neutralized in place as with formaldehyde and it does not require post-exposure wipe down. As a gas it easily flows through HEPAs for complete decontamination. Chlorine dioxide gas can be direct-vented (Federal, state, and local laws must be followed) or scrubbed out. Two types of scrubbers are available: a carbon packed scrubber and a sodium thiosulfate counter-current scrubber. Carbon scrubbers are simple boxes containing carbon with the gas flow going through the carbon bed which absorbs the CD gas (Wood, 2010). With sodium thiosulfate, the gas stream is introduced into the bottom of a "scrubber" and the solution of sodium thiosulfate is sprayed over the gas stream rising from the bottom.

Two methods for the decontamination of BSCs using CD gas have been validated by NSF. The CD gas validation studies were performed in all class II cabinets (A1, A2, B1, and B2) from various manufacturers (Luftman, 2008). The following two methods for chlorine dioxide gas decontamination are paraphrased from NSF 49 Annex G Part 7.

Method 1 utilizes a fixed amount of CD gas. This method is similar to using formaldehyde as the volume of the cabinet determines how much CD gas must be generated. It can be generated by creating a concentrated solution of chlorine dioxide and allowing it to off-gas by bubbling or effervescence, or it can be generated by

passing dilute chlorine gas over a cartridge of sodium chlorite that converts the chlorine to pure chlorine dioxide. Such systems are available from DRS Labs (Lehigh, PA) and ClorDiSys Solutions (Lebanon, NJ). Once this amount is generated, the exposure time is started. If the concentration drops during the process, no make-up gas is provided. A safety factor was added to account for the loss of gas due to reaction with loaded HEPAs. As with formaldehyde, the cabinet must be sealed. Once the cabinet is sealed, the RH is raised to 60%-85% by using a small steam generator, ultrasonic humidifiers, or a hot plate and some water. The cabinet blower or a recirculation system must be running during humidification. Once the RH is at proper levels, the CD gas can be introduced, either through a front connection plate or by placing the generator inside the BSC, to reach 0.13 g/ft^3 (4.7 g/m^3). The cabinet blower or recirculation must be running during the gas-generation process. After the generation process is complete, the cabinet blower should be energized for 1 minute every 15 minutes of contact time. The CD exposure or contact time is 85 minutes. After this time has elapsed, the CD gas is removed from the cabinet by opening the duct damper if it exists or energizing the scrubber to remove the gas. The ducted cabinets can be aerated in 5-10 minutes compared to 20-30 minutes for scrubbers. Using low-level safety sensors ensures the cabinet is below the short-term exposure level (STEL) of 0.3 ppm before removing the cabinet sealing.

Method 2 utilizes a fixed concentration of chlorine dioxide gas. This method requires the capability of monitoring the concentration and the ability to add more concentration if needed. The CD gas is generated by passing dilute chlorine gas over sodium chlorite cartridges to reach a specific concentration (3 mg/L [1086 ppm] or 5 mg/L [1810 ppm]) and is maintained throughout the entire exposure time (60 minutes for 3 mg/L; 45 minutes for 5 mg/L). This method requires equipment that is capable of generating the gas on demand, monitoring the gas, and maintaining the gas concentration. If the CD gas concentration drops during the exposure time, then more gas must be added. The sealing method, RH requirements, and aeration are the same for Method 2 as described above in Method 1. The differences are that a specific concentration is reached, and just as importantly, the fixed concentration is maintained. This requires either automatic generators (ClorDiSys Model Minidox-M or Cloridox-GMP [ClorDiSys Solutions, Lebanon, NJ]) or the user to maintain control over the process by measuring the CD gas concentration levels and maintaining them.

Vapor Phase Hydrogen Peroxide (VPHP)

Hydrogen peroxide vapor has been used for years in chambers such as isolators (Akers, 1995), rooms (Meszaros, 2005), and facilities (Herd, 2005). Benefits of vapor phase hydrogen peroxide (VPHP) are that it leaves no residues as compared to formaldehyde, and that it is considered by most to be non-carcinogenic. IARC, NTP, and OSHA do not list hydrogen peroxide as a carcinogen. ACGIH lists hydrogen peroxide as an A3 animal carcinogen. The vapor is generated by boiling/vaporizing 31%-35% liquid hydrogen peroxide to generate the VPHP. Currently, there are two different processes for VPHP decontamination: the wet process and the dry process. The dry process requires no condensation while the wet process is designed to promote "microcondensation." Either way (wet or dry), it is difficult to eliminate condensation for the dry process and it is impossible to obtain uniform condensation with the wet process because of the varied surface materials and other environmental factors such as temperature and relative humidity (Hultman, 2007). VPHP can be direct-vented or converted as a method of aeration. Conversion usually involves some metal (silver, platinum, or galvanized steel [Carlsen, 2005]) that catalytically converts the VPHP to oxygen and water.

While VPHP is not a recommended method of decontamination by NSF, several articles and publications describe the process to achieve decontamination of BSCs. The following paragraphs discuss the cycles developed, the processes used, and the validation process utilized.

Esco Technologies. Inc. (Marlboro, PA), one of the many manufacturers of BSCs, has performed studies with both processes (wet and dry) to decontaminate a cabinet (Lin, 2010a). The wet process utilized the Infinity Class II type A2 6-ft cabinet. Successful runs required 80 minutes of gassing time plus 4 hours and 10 minutes for aeration for a total decontamination cycle time of 5.5 hours (Table 2). With this particular vapor generator system (Bioquell EBDS, Horsham, PA), the vaporizer was placed inside the BSC work area. An external blower was then connected from the top of the BSC and recirculated back into the workspace of the BSC. The BSC blower was turned off during the process. When the exposure step was completed, the aeration phase started by turning on the catalyst inside the BSC and also using an external catalyst in the recirculation loop.

Esco also performed studies using the dry process in the same Class II type A2 6-ft cabinet as the previous studies (Lin, 2010b). With this system (Steris VHP-1000 ARD, Steris Corporation, Mentor, OH) successful cycles were demonstrated with a 4-hour cycle time. With this process, the generator was placed outside the BSC and the VPHP was injected into the work area of the BSC (the VPHP concentration was not noted). The VPHP return was taken from the exhaust HEPA on top of the BSC. Table 3 outlines the specific cycle parameters used for the Steris cycles (see Steris VHP 1000 ARD operators' manual for parameter description). There are Esco Technologies, Inc. manufacturer-suggested cycles for other 3-ft, 4-ft, and 5-ft cabinets, but these cycles were not validated.

Another BSC manufacturer, The Baker Company

(Sanford, ME), has performed runs to test the VPHP dry process (Jones, 1993a). This study was performed in a Baker Model SG-600 Class II Type A2 BSC using the Steris VHP 1000 VPHP generator. Several runs were performed and changes were made to the BSC system so the concentrations could build quickly. An additional flow channel was installed, connecting the plenum above the exhaust filter to the supply blower plenum. After many runs the cycle parameters were determined and five validation runs were performed. Table 4 outlines the cycle parameters used for the Steris cycles (see Steris VHP 1000 operators' manual for parameter description).

Total decontamination cycle time was 301 minutes. VPHP concentration during decontamination was 2.75 mg/L. From its studies, Baker determined that VPHP is an effective alternative to formaldehyde, but only if the appropriate cycle parameters are determined. It was also specified that each BSC model is different and each must go through the cycle development process.

Another study that evaluated BSC decontamination with VPHP was done with the wet process on two different BSCs: Baker Model VBM-600 Class II type B1 and NuAire NU-440-400 Class II type A2 biological safety cabinets (Plymouth, MN) (Driskill, 2007). These studies were similar to the other wet process studies with the vaporizer inside the work area of the BSC and an external recirculation loop set up from the exhaust HEPA to the front face of the BSC. This study used 12-16 BIs per run. Initial studies found repeatable failures in the corners of the BSC. To rectify the problem of having positive BIs the BSC blower was pulsed for 8-12 seconds twice during the gassing cycle: at 20 minutes and at 40 minutes. The overall concentration obtained inside the BSC was not indicated. The cycles that were used are described in Table 5 (see Clarus TM "S" Hydrogen Peroxide Vapor Generator operators' manual for parameter description [Salt Lake City, UT]).

A slight leakage was evident on the front side of the

Table 2
Bioquell VPHP Cycle Parameters (Lin, 2010a)

Width (ft)	Manufacturer	"Set" Volume (ml)	Gassing Time	Aeration Time
6	Esco	155	80 minutes	4 hours 10 minutes

Table 3
Steris Cycle Parameters (Lin, 2010b)

Process	Time (minutes)	Airflow (SCFM)	Injection Rate (g/m)
Dehumidification	15	20	—
Conditioning	4	17	6.0
Decontamination	45	17	3.5
Aeration	180	20	—

Table 4
Steris Cycle Parameters (Jones, 1993a)

Process	Time (minutes)	Airflow (SCFM)	Injection Rate (g/m)
Dehumidification	60	12	—
Conditioning	1	12	6.7
Decontamination	30	12	3
Aeration	210	12	—

Table 5
Bioquel VPHP Cycle Parameters (Driskill, 2007)

Width (ft)	Manufacturer	"Set" Volume (ml)	Gassing Time	Aeration Time
4	Nuaire	155	80 minutes	70-130 minutes
6	Baker	155	80 minutes	70-130 minutes

Baker cabinet on two of the cycles. The readings were between 0.1-2.0 ppm. With the particular model VPHP generator (Bioquell Clarus "S"), the pressure in the cabinet should have been negative thereby reducing the concerns for leakage, but there was still some leakage. A safety sensor was used to immediately find the leakage and correct the issue. The issue was attributed to a dried out seal on the front of the cabinet.

Another study with VPHP was done with the VPHP generator Bioquell Clarus L using the wet process (Hillman, 2004). This study was done by the BSC manufacturer NuAire Inc. on a NuAire (NU-440-400) Class II type A2 cabinet. The successful process had a conditioning time of 20 minutes. This step brought humidity levels to 30%. The time the VPHP was injected (gassing time) was not noted. It had a dwell or exposure time of 40 minutes and finally an aeration or removal time of 180 minutes to reach a chamber concentration of 8 ppm. An additional 3 hours (6 hours total aeration) was required to reach the OSHA PEL of 1 ppm. During this testing there were repeatable BI failures in the pleats of the HEPA filters. To achieve successful decontamination of the BSC, the blower had to be cycled for 15-20 seconds every few minutes during the gassing stage. During the dwell or exposure stage, the BSC blower was cycled only once. The total decontamination time was 9 hours including the aeration time; if the cabinet is ducted, the cycle time could be reduced to about 5 hours.

Discussion

When performing any decontamination—whether in a BSC or room—the user must evaluate what is necessary for an effective decontamination. What must be recognized is that all decontamination methods can work if the agent reaches ALL surfaces for a prescribed amount of time, at the correct concentration. This means it must have: 1) good and complete distribution; 2) thorough and total penetration; and 3) sufficient contact time at specified concentration. Any decontamination method requires a complete and thorough distribution of the sterilant to get an effective decontamination. Specifically, the agent used must get to all corners of the work area in the BSC, it must get below the work surface, it must get to the plenums, it must get through the HEPAs, and it must get to the clean side of the exhaust HEPA. Are the HEPA filters loaded or clean? This can affect decontamination. Loaded or dirty filters will absorb or react with each sterilant. CD was validated by NSF with worst-case loaded/dirty HEPAs which can be an issue with VPHP. VPHP also has issues with soiled loads (Krishnan, 2006). Furthermore, are the HEPA filter frames wood or aluminum? Wood or cellulosic materials readily react with VPHP (JSPPTOH, 2004; Jones, 1993B; Ryan, 2006; SAIC, 2005). What is the ambient temperature or humidity (starting RH levels)? With formaldehyde

and chlorine dioxide gas, RH is typically brought to 60%-75%. Moisture is critical for all spore log reduction independent of the agent used (formaldehyde, CD gas, or VPHP) (Agalloco, 2008; Westphal, 2003; Whitney, 2003). With VPHP in the dry process, studies show reduction of humidity prior to hydrogen peroxide injection (Jones, 1993a; Lin, 2010b). Studies also show the reduction of RH is not necessary (Driskill, 2007; Lin, 2010a), which is referred to as the wet process. The wet process relies on a different application method (condensation) than the dry process and both processes are considered different. Regardless of the VPHP process used, both inject humidity or water during the process. In the dry process the injection of humidity is solely due to the vaporization of the H_2O_2 solution. Are there any cold spots on the BSC? Formaldehyde can condense on cold surfaces thereby reducing the overall concentration within the chamber, which may have an adverse effect on the cycle (Ackland et al., 1980). VPHP can condense on cold surfaces also reducing the concentration in air within the chamber. In the dry process this can be an issue, but the wet process requires condensation.

Cold spots can easily occur from an air-conditioning supply duct or vent placed near a BSC. When the air conditioning turns on, it can cause a cold spot below the vent. Cold spots can also occur if the BSC is placed near an outside wall and the temperature outside is cold. This may lead the user to select CD since it does not condense or the VPHP wet process since condensation is desired. Another question that must be asked: Is the cabinet ducted or not ducted? Ducted cabinets will drastically decrease the aeration time for all methods. It can be as fast as 5-10 minutes with formaldehyde or chlorine dioxide gas based upon 300 CFM exhaust rate and 85 ft[3] BSC volume and can be 30 minutes for VPHP (Lin, 2010b). Ducted cabinets also bring up concerns for the damper that isolates the BSC. Is the damper sealed? If the damper is not fully closed or sealed, the concentrations will drop in the BSC and therefore cause an ineffective decontamination cycle unless the concentration is being monitored and maintained as in Method 2 of NSFs validation of chlorine dioxide gas.

Conclusions

Space decontamination is mandatory when maintenance work, filter changes, and performance tests require access to any contaminated portion of a BSC (NSF/ANSI 49 Annex G). BSCs must be decontaminated prior to decommissioning and salvage and before physically moving the cabinet (NSF/ANSI 49 Annex G). In most instances where BSC decontamination is necessary, one of the procedures (depolymerized paraformaldehyde or chlorine dioxide gas) should be used (NSF/ANSI 49 Annex G). Prior to decontamination with an alternative method (such as VPHP), cycle parameters and

validation of those parameters must be developed for each manufacturer, model, and size of BSC (NSF/ANSI 49 Annex G).

When considering decontamination methods, many factors must be taken into account: safety, cycle time, effectiveness, equipment costs, NSF requirements or NOT, etc. Formaldehyde equipment can cost $20-$10,000. VPHP generators have a cost range from $39,000-$100,000, and CD generators can cost $5,000-$105,000.

Gases like formaldehyde and chlorine dioxide provide the best penetration and distribution. Chlorine dioxide is not a carcinogen, while formaldehyde is considered by most to be a human carcinogen and hydrogen peroxide is considered by some to be a class A3 animal carcinogen. CD and VPHP both provide a residual-free chamber. NSF-recommended cycles are developed for both formaldehyde and chlorine dioxide but need to be developed for VPHP. The constant theme in the VPHP articles was the importance of developing cycle parameters because these differed from study to study. Most of the cited references validated cycles in Class II type A2 cabinets, with one reference using a Class II type B1 cabinet. Material compatibility must also be considered. Formaldehyde has the best material compatibility, while VPHP and CD gas are both oxidizers. Most BSCs are not decontaminated frequently, so the oxidizing effects of both VPHP and CD are not an issue.

Cycle times for each process differ. Formaldehyde is typically a 2-day process. Day 1 consists of setup and generation of the formaldehyde gas, and Day 2 involves the neutralization process. With chlorine dioxide gas, the cycle time is greatly reduced to 45-minute, 55-minute, or 85-minute exposures. The total cycle time for chlorine dioxide gas has been 3-4 hours including setup, decontamination, aeration, and break down. With VPHP the process varies depending upon the study. Table 6 outlines the different cycle times.

Regardless of the method used, all cabinets must be sealed. The importance of this is demonstrated in the Driskill study (Driskill, 2007) where some VPHP leakage was evident. All agents are deadly and kill organisms (humans included), or they would not be used to decontaminate.

Acknowledgments

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Table 6

VPHP Cycle times, including setup, decontamination, aeration and breakdown.

Reference	Total Cycle Time (hours)
Lin, 2010a	3 ducted, 6 non-ducted
Lin, 2010b	5
Jones, 1993	6
Driskill, 2007	4-5
Hillman, 2004	5 ducted cabinet, 9 non ducted

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Presidential Bioethics Panel Releases Report

In the U.S., the President's Bioethics Commission released a report that said additional regulations for the field of synthetic biology are not required at this time. The recommendations include self regulation by synthetic biologists, as well as "an ongoing system of prudent vigilance that carefully monitors, identifies and mitigates potential and realized harms over time." The report, press release and other information is on the Commission's web site at www.bioethics.gov/