Chlorine Dioxide Gas is Now Approved for the Decontamination of Biological Safety Cabinets Under Annex G of NSF/ANSI 49

Introduction

Formaldehyde had been the only fully accepted process for Biological Safety Cabinet (BSC) decontamination by NSF International under their NSF / ANSI 49 standard until recently. When formaldehyde was approved in 1983, Chlorine Dioxide gas and vaporized hydrogen peroxide were still in their beginning stages as decontaminating agents. Since formaldehyde's approval, no other method has been approved until now. Chlorine Dioxide gas has now been named as an appropriate method for the decontamination of BSCs. Although formaldehyde is easily circulated within the cabinet, has fairly good penetrability, and has the proper sporicidal activity, there were enough disadvantages to warrant the search for a new method. As such, in 2005 NSF International established a task group to design and implement a protocol to validate the use of Chlorine Dioxide gas, and to revise the NSF / ANSI 49 standard for BSCs.

Reason for Change

With formaldehyde, a residue is typically left following a decontamination, which can be neutralized from easily accessible areas using an ammonia solution. However the residue is much harder to remove from hard to reach places, such as blowers, HEPA filters and plenums. The ammonia solution can also leave a residue if too much is used, making a residual wipe down necessary as well. Formaldehyde is also considered to be a carcinogen in much of the world. This makes its use and residual levels a concern to those involved with the decontamination of BSCs and those working inside of them also.

Chlorine Dioxide gas was selected for testing due to its penetrative properties as a gas similar to formaldehyde. Chlorine Dioxide gas can be easily distributed throughout the cabinet and through HEPA filters, decontaminating them as well because of its good penetrability. Vapor systems have difficulty penetrating and decontaminating the HEPA filters, due to its propensity to condense inside the cabinet. Chlorine Dioxide gas also has the proper sporicidal activity, giving it all of the benefits that formaldehyde had. Chlorine Dioxide gas however, does not share formaldehyde's downsides of residues left in the cabinet, or its carcinogenicity. NSF International recommends a 6 hour gas exposure when using formaldehyde, which does not include time necessary for aeration or cleaning up residues left in the cabinet. A complete decontamination cycle of a BSC using Chlorine Dioxide gas can be performed in 90 minutes from start to finish.

Methods and Testing

The task group decided that, like formaldehyde, it was not necessary to validate the effectiveness of Chlorine Dioxide gas in each model and size of BSC because Chlorine Dioxide gas is a non-condensing gas at room temperature. It was deemed sufficient for validation to test Chlorine Dioxide gas on at least two different makes of Class II type A1 and A2 bench and console models, B1 and B2 biological safety cabinets. Three successful decontamination runs on each cabinet needed to be performed to validate the process. This accounts for a total of 8 individual BSCs needing to be tested successfully for validation.

In addition, two methods of Chlorine Dioxide gas decontamination were performed. The validation of Method 1 was performed at the NuAire, Inc. facility in Plymouth, MN. The BSCs used were NuAire models S602-600 (type A2 console, 6ft, 2.11m^3 volume), NU437-400 (type A2 bench top, 4-ft, 1.28ft^3 volume), NU430-600 (type B2, 6-ft, 1.94m^3 volume), NU427-400 (type B1, 4-ft, 1.55m^3 volume), and NU427-600 (type B1, 6-ft, 1.94m^3 volume). Method 1 involves the introduction of a fixed amount of Chlorine Dioxide gas into the BSC, proportional to the volume of the BSC. This amount of Chlorine Dioxide gas would be held for a fixed amount of time. 3.5 g of Chlorine Dioxide gas per cubic meter (3.5g/m^3) would be held for 80 minutes. This method is similar to the current formaldehyde procedure, which calls for a measured quantity of paraformaldehyde to be placed inside the BSC for decontamination. The concentration of Chlorine Dioxide gas would not be measured during the decontamination cycle, also in accordance to the current formaldehyde decontamination practices. This method was tested in order to provide an almost exact replacement of the current formaldehyde procedure.

Method 2 was validated at Micro-Clean, Inc in Bethlehem, PA. BSCs manufactured by Baker, Inc were used for validation of Method 2. Models used were a B60-112 (type A1, 6ft, 2.0m^3 volume), SG-403 (type A2, 4-ft, 1.4m^3 volume), NCB-B6 (type B1, 6-ft, 2.7m^3 volume), and a 4-TX (type B2, 4-ft, 1.4m^3 volume). Method 2 involves introducing Chlorine Dioxide gas until a specified concentration has been reached. The concentration level would then be monitored throughout the entire cycle, with additional Chlorine Dioxide gas introduced if the concentration fell due to leakage, absorption, or decomposition. In addition, two separate concentrations of chlorine dioxide gas would be generated and maintained, to give flexibility to the process and the user. The concentrations used were 4.8 mg/L and 2.8 mg/L. The different concentrations were coupled with different exposure times, with the 4.8 mg/L concentration being held for 40 minutes, and 2.8 mg/L of Chlorine Dioxide gas being held for 55 minutes.

Method 2 requires an automated Chlorine Dioxide gas generator with a concentration monitor in order to check and maintain the concentration. However method one does not need a generator with a concentration monitor in order to be performed. Validating these two methods allowed for increased flexibility for the process, leaving more options for those using Chlorine Dioxide gas when decontaminating BSCs. The availability of Method 2 allowed the benefit of a slightly shorter cycle time and the use of automated equipment.

Efficacy Testing

Biological Indicators were placed within the cabinet to test the efficacy of the decontamination cycles. They were placed in pairs at each predetermined location. Biological Indicators were placed in what was considered to be the most challenging positions for gas decontamination. A total of 12 Biological Indicators were placed within each BSC during each validation run. Three pairs were placed on the clean side of the exhaust HEPA filter. One pair was placed in the BSC's plenum, another pair on the dirty side of the supply HEPA filter, and the last pair underneath the BSC workspace. None were placed in the workspace since this was determined to be the easiest place for kill. It was decided to use *bacillus atropheus* spores on paper substrates, to keep in line with what was used for most of the work to validate formaldehyde. *Bacillus atropheus* is the preferred indicator organism for biological indicators when testing the efficacy of gaseous decontamination agents. The target value for decontamination within the cabinet was a 6-log (99.9999%) reduction of spores. Validation of a method on a particular cabinet required at least 3 successful decontamination cycles. A cycle was deemed successful if all six test locations for biological indicators passed. Further challenging the validation cycles was the fact that the HEPA filters were loaded with soil. Soil was added until there was a 50% pressure drop across the HEPA filter. This would demonstrate a worst-case scenario when BSCs required decontamination.

Conclusion

Testing proved successful as Chlorine Dioxide gas passed all requirements set forth in the validation protocol by NSF International. Type A and Type B BSCs were used in the validation study, both being shown to be compatible with Chlorine Dioxide gas and its decontamination process. Three decontaminations were performed in each cabinet to validate the process for efficacy for a total of 36 successful test runs. No residues were evident within the BSCs after the decontamination cycles were performed. In all trials, no material degradation was noticed within the BSCs used.

Chlorine Dioxide gas was shown to be effective in penetrating and decontaminating soiled HEPA filters, as well as the entirety of the Biological Safety Cabinet. Chlorine Dioxide gas was proved to effectively decontaminate Biological Safety Cabinets, without any of the negative aspects of using formaldehyde. As such, Chlorine Dioxide gas is now approved under Annex G of NSF/ANSI 49 to be used in the decontamination of Biological Safety Cabinets.

Additional Information

Annex G of NSF/ANSI 49 allows for two different methods of using Chlorine Dioxide gas in the application of decontaminating BSCs. Method 1 consists of introducing a set amount of $0.13 g/ft^3$ of Chlorine Dioxide gas and having an exposure time of 85 minutes. Method 2 involves injecting and maintaining a set concentration level for a particular amount of time. Two concentrations were approved, with a concentration of 5.0 mg/L being maintained for 45 min, and a concentration of 3.0 mg/L being maintained for 60 minutes. Concentration levels and exposure times approved for use have been increased from those used during validation testing by NSF International to include an added safety factor.