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THE EFFECTIVENESS OF GASEOUS FORMALDEHYDE DECONTAMINATION ASSESSED BY BIOLOGICAL MONITORING

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ABSTRACT

The results of seven years experience of the effectiveness of routine gaseous formaldehyde decontamination of rooms, filter canisters and biological safety cabinets at ambient temperature are reported. The effectiveness of each decontamination was monitored biologically by the inactivation of *Bacillus stearothermophilus* spores at five locations in rooms and biological safety cabinets and at two locations in filter canisters. A greater than 10^4 reduction in viability was achieved for each group of five spore tests in 92% of room decontaminations and each group of five spore tests in 81% of biological safety cabinet decontaminations, and a greater than $10^{4.7}$ reduction for both spore tests in 83% of filter canister decontaminations. This long-term study of 2,308 routine formaldehyde decontaminations of varying spaces in practical situations demonstrates the reliability of the procedure. The results confirm that previously-described methods can be applied with confidence for routine decontaminations of laboratory facilities and equipment.

INTRODUCTION

Formaldehyde gas has been used successfully for decades to decontaminate laboratory facilities and equipment. Earlier reports generally addressed some of the critical gaseous treatment parameters such as the method of generation of formaldehyde (Taylor et al., 1969), the ability to penetrate materials and crevices (Hoffman & Spiner, 1970; Spicher & Borchers, 1983) and temperature, time and relative humidity (Ackland et al., 1980; Songer et al., 1984). Several investigators have subsequently explored alternate methods of formaldehyde generation (Coldiron & Janssen, 1984; Green & Kulle, 1986) and means of monitoring its concentration during cycles (Green & Kulle, 1986; Coyne et al., 1986). Suzuki & Namba (1982) have reported results of routine formaldehyde decontamination cycles of surgical instruments in small laboratory cabinets using extended periods of exposure. However, the concentration of formaldehyde used in the latter procedures was variable and so was not measured.

As the complexities required for effective formaldehyde decontamination of microorganisms became widely appreciated, reports were published that also covered aspects such as the need to disperse the gas to establish uniform concentration levels within spaces to avoid condensation of formaldehyde (Lach, 1990), and considerations of methods to monitor biologically the efficacy of decontamination procedures (Cross & Lach, 1990).

Previously published procedures have described methods for the generation of formaldehyde and its monitoring only in simulated laboratory situations (Green & Kulle, 1986; Favero &

Bond, 1991). Methods used by Songer et al., (1972), Ackland et al., (1980) and Lach (1990) could be extended for practical use in large volumes such as rooms. However, reports which describe the results of routine formaldehyde decontaminations of large spaces have not been published to our knowledge. Scaling up procedures from model systems often does not produce predicted results. Similarly, routine application of experimental methods may not produce desired reliability. Thus, the results of this long-term application of gaseous formaldehyde decontamination methods will provide new information about its reliability.

This paper describes the methods used for the decontamination of high efficiency particulate air (HEPA) filter canisters (0.8 cu. m), laboratory rooms (up to 325 cu. m), and Class II biological safety cabinets (1.7 cu. m). Results of decontaminations over six, seven or eight year periods, respectively, are expressed in terms of biological inactivation of *Bacillus stearothermophilus* spores.

METHODS

Decontamination of Room Spaces

Formaldehyde decontaminations were done in 37 rooms or dedicated decontamination chamber air locks with volumes ranging from 19 to 325 cu. m. Most decontaminations were done in room spaces with volumes from 27 to 100 cu. m. Rooms and chambers were able to be completely sealed and wall, ceiling and floor surfaces were impervious to formaldehyde. Prior to decontamination, all surfaces were cleaned to remove visible dirt, supply ducts were closed and exhaust ducts left partly open to provide pressure control. The temperature of the building was maintained between 21°C and 24°C without humidity control.

Formaldehyde gas was generated by sublimation of paraformaldehyde at a concentration of 5 g/cu. m room space in an electrically heated 41 pot containing silicone oil at 160°C. Sublimation was allowed to proceed for two hours with mixing of the air and gas by the use of oscillating fans. A volume of water calculated to produce a relative humidity of 70% to 90% was boiled simultaneously. Calculated volumes, deduced from psychrometric charts and modified by experimental experience, were used for three ranges of ambient humidity, depending on climatic conditions at the time. Decontamination was allowed to proceed for at least 15 hours at temperatures that reached as high as 30°C for short periods during vaporization of gases. In most cases, neutralization of gaseous formaldehyde was achieved by heating solid ammonium carbonate (7.5g/cu. m room space) at 120°C in silicone oil, prior to purging the gases from the room. In other cases, the formaldehyde gas was purged from the room at the end of the decontamination cycle and ducted to a water scrubber. Frequently used decontamination chambers were monitored by electronic detectors measuring formaldehyde gas concentration and relative humidity. The electronic data output was collected, stored on computer and transformed into graphic display in the central monitoring station. In other rooms, relative humidity was measured by thermohygrograph and residual formaldehyde concentration was measured by hand-held meters at the completion of the decontamination process, ensuring a safe atmosphere for staff to reenter the area.

Biological monitoring of the effectiveness of the decontamination was traced by the use of 10^4 spores of *Bacillus stearothermophilus* (derived from a commercial preparation of ATCC 7953) dried onto 40 mm x 10 mm sterile aluminum strips, and exposing the strips in five selected room locations (one on each wall and one on the floor) immediately before generation of the formaldehyde. One uninoculated control strip was included as a control. Following completion of the cycle and purging of the gases, spore strips were collected aseptically into separate, sterile, labeled vials for return to the laboratory for testing. Sterile tryptone soya broth medium (OXOID CM129, 10 ml) was added aseptically to each vial, mixed oil a vortex mixer for 10 seconds, and incubated at 56°C for seven days with agitation and visual examination of the vial on at least four intermediate days. With each set of five test strips, an unexposed positive control was included; growth of *B. stearothermophilus* was determined by cloudiness of the medium.

Decontamination of Canisters for HEPA Filters

At this laboratory, exhaust air from all work rooms was filtered through two HEPA filters in series. Each filter was housed in its own cylindrical canister of volume 0.8 cu. m. The filters were isolated by valves between filter canisters and the ductwork. Decontamination pipe stubs and valves allowed a purpose-built formaldehyde generation rig including a side channel blower air fan to be connected to the isolated zone to ensure circulation and uniform mixing of gases within the canister.

Formaldehyde gas was generated by boiling formalin solution (30 ml). Additional water (25-40 ml) was included prior to boiling to ensure a final relative humidity >70% was achieved. Decontamination was allowed to proceed for 15 hours. Initially, the temperature would have been above the ambient temperature of 21-24°C because of the heat generated during vaporization of the liquids. After the decontamination cycle, gases were purged from the canisters by air, and any residual formaldehyde neutralized by vaporizing ammonium carbonate (20 g) generated in a separate, but similar, purpose-built rig.

Biological assessment of the effectiveness of the decontamination was followed with two spore strips (prepared as described above except that 5 x 10^4 spores per strip were used) placed in a port on the downstream side of the filter canister. The higher spore number was used because better inactivation was observed to occur in the spore strip placed in the return decontaminating gas duct; $10^{4.7}$ spores on the downstream side equated to a kill of 10^4 spores on the strip placed on the upstream side of the filter. Recovered spore strips were tested for viable cells by the procedure described above.

Decontamination of Class II Biological Safety Cabinets

After clearing ancillary equipment from cabinets, all surfaces and the sump were cleaned with a disinfectant. Thirty-eight ml formalin (37-40% formaldehyde solution) was vaporized by boiling in an electrically heated pan with 5-15 ml water (depending on ambient humidity) to produce a formaldehyde concentration of 8.5 g/cu. m and relative humidity of 70-90% at the ambient temperature of 21-24°C. The cabinet front was closed with the manufacturer's

decontamination panel and the joints sealed with adhesive tape. The ambient relative humidity was measured by a whirling psychrometer and the process relative humidity by a thermohygrograph. Plastic lay-flat tubing formed a duct between one of two exhaust decontamination panel spigots and the room exhaust-air spigot which could be closed with a tie. A second tube formed a re-circulating duct from the second exhaust decontamination panel spigot to the front decontamination panel spigot.

Formaldehyde gas was generated and circulated for one hour by the cabinet fans. The formaldehyde was allowed to dwell for 15 hours before being vented by opening a port on the front panel to admit room air which was exhausted by the cabinet exhaust fan via the room exhaust spigot. Residual formaldehyde was neutralized by generating ammonia gas from 20 g of ammonium carbonate heated for 10 minutes at 120°C. The exhaust duct was tied and the re-circulating duct opened to allow re-circulation of ammonia for 20 minutes prior to purging from the cabinet for 15 minutes in as manner to the formaldehyde gas. Biological assessment of the effectiveness of the decontamination was as described for room spaces above.

RESULTS

Over a seven year period, rooms or large spaces were decontaminated on 1,513 occasions using the procedure described. The effectiveness of the decontamination was measured by residual viability of 10^4 *B. stearothermophilus* spores following incubation for seven days at 56°C.

TABLE 1
Spore Test Results following Formaldehyde Decontamination of Rooms and Chambers

Year	Number of decontaminations	Number of tests with 1 test spore strip positive	Number of tests with ≥2 spore strips positive
1987	204	2	9
1988	238	12	23
1989	268	5	9
1990	227	5	3
1991	194	1	2
1992	190	5	16
1993	192	17	11
TOTAL	1,513	47	73

Total inactivation of spores on all five test strips occurred in 92% of cases. Those in which either one, two or more of the test spore strips showed growth are shown in Table 1.

Over a six year period, HEPA filter canisters were decontaminated with formaldehyde on 604 occasions. The effectiveness of decontamination was determined by measuring residual

viability of 5 x 10^4 *B. stearothermophilus* spores following incubation for seven days at 56°C. Two test spore strips were included in each decontamination. In 8.2% of experiments, both test spore strips showed thermophilic bacterial cell growth characteristic of *B. stearothermophilus*, indicating the decontamination was incomplete (Table 2). Of the 50 experiments in which this occurred, 35 were done in the winter and spring months from June to October when room temperature and relative humidity were at the lower end of usual values.

TABLE 2
Spore Test Results following Formaldehyde Decontamination of HEPA Filter Canisters

Year	Number of decontaminations	Number of tests with 1 test spore strip positive	Number of tests with 2 spore strips positive
1988	69	9	9
1989	74	9	7
1990	81	3	2
1991	103	0	1
1992	118	5	16
1993	159	27	15
TOTAL	604	53	50

Over an eight year period, Class II biological safety cabinets were decontaminated on 191 occasions as described. The effectiveness of the decontaminations was determined by measuring the residual infectivity of 10^4 *B. stearothermophilus* spores after incubation for seven days at 56°C. In 6.8% of tests, one of the five test spore strips retained infectivity and in 11.5%, two or more spore strips retained infectivity (Table 3).

TABLE 3
Spore Test Results following Formaldehyde Decontamination of Class II Biological Safety Cabinets

Year	Number of decontaminations	Number of tests with 1 test spore strip positive	Number of tests with 2 spore strips positive
1986	17	5	2
1987	17	2	4
1988	26	2	0
1989	18	1	1
1990	26	0	6
1991	34	0	2
1992	25	2	4
1993	28	1	3
TOTAL	191	13	22

DISCUSSION

As only cleaned surfaces and equipment only were subject to decontamination in this report, the inactivation of gross contaminants that might be expected in other circumstances was not attempted. Effective decontamination with gaseous formaldehyde requires close control of a combination of parameters including gas concentration, temperature, relative humidity and exposure time (Cross & Lach, 1990). The combination of parameters used at this institute was determined following extensive testing from 1984-1986, and was intended to meet internal requirements for the inactivation of animal pathogens, usually viruses.

Methods for testing the efficacy of decontamination procedures must be precise and reproducible when using test organisms. Such procedures are often referred to as "low," "intermediate" or "high" decontaminations based on the category of test organisms used (Favero & Bond, 1991). Such tables show bacterial spores have a higher resistance to germicidal chemicals than any other category of microorganism (prions excepted). Spores of *Bacillus* species fit experimental requirements for stability and consistency, and because of their strong resistance to chemical and physical inactivation, are ideal for the standardization of such tests (Cremieux & Fleurette, 1991). *B. stearothermophilus* (rather than other types of Bacillus spores) was chosen as the indicator organism because of its known high resistance to inactivation by formaldehyde and its high optimal temperature for growth which precludes the replication of most other microorganisms and so reduces the probability of false positive growth as a result of accidental contamination. As titration of the residual spores on each strip was not done, cloudiness of the medium was presumed to represent growth of *B. stearothermophilus.* Tests to identify the growing organism were not done routinely. However, swab cultures of decontaminated surfaces were done occasionally to ensure that ubiquitous bacteria were totally inactivated by the procedure.

For a trial period of several months, growth was detected by acid production (as shown by color change of the bromothymol blue indicator) in an alternative medium (spore strip broth, OXOID CM 763). However, growth in this medium was observed less frequently and the medium was shown to be less sensitive.

Because the efficiency of initiating infections is so low for most viruses, failure to recover virus following an inactivation test does not necessarily indicate a 100% kill. Thus, no methods for testing decontamination procedures for viruses have been generally adopted (Cremieux & Fleurette, 1991), although a variety of such tests have been described (Chen, 1991). The mechanisms of virus inactivation by chemical decontaminants follow an exponential pattern, in most cases, and so resemble the death course of bacteria (Sykes, 1965). Klein (1965) showed that the three oldest germicides (5% phenol, 200 ppm sodium hypochlorite and 70-95% ethyl alcohol) were all effective in inactivating members of all major virus groupings. Although some bactericides are not effective against all viruses, there has been a general acceptance that viruses are not significantly more resistant to chemical decontaminants than bacteria (Cremieux & Fleurette, 1991; Chen, 1991; Sykes, 1965) and certainly less resistant than bacterial spores.

For most purposes at this laboratory, sterilization by formaldehyde was not required, but rather an inactivation of the small bioburden of animal pathogens of concern remaining in laboratory zones and equipment after thorough cleaning. In most cases, these agents of concern were viruses. As none were known to be more resistant to formaldehyde than *Bacillus* spores, conditions providing at least a 10^4 reduction in *B. stearothermophilus* spores provided sufficient confidence to conclude that any animal viruses present would have been inactivated by the treatment.

The results of the practical use of formaldehyde decontaminations over a long trial period indicated that reproducible inactivations could be achieved. Therefore, spaces and surfaces routinely exposed to formaldehyde were initially assumed to be effectively decontaminated on the basis of quality control of the processes of humidity, formaldehyde concentration and dwell time from the central monitoring computer. Biological confirmation of this data was provided by the lack of growth of spores and was not finalized until after seven days of incubation. However, the interpretation of the inactivation results was not always definitive and such difficulties have been noted by others (Cremieux & Fleurette, 1991). Gross failure of the decontamination process was detected at 18 hours if any spore strips were positive. In a very small number of cases, all spore strips showed bacterial growth, and these cases were invariably linked to a mechanical or procedural fault such as the failure of the heating unit to disperse either the paraformaldehyde, or the water to elevate the relative humidity. In such cases, the error could be detected immediately and the decontamination procedure repeated. Such explained failures occurred occasionally but are not included in the data shown in Tables 1-3.

A qualitative measure of the effectiveness of the formaldehyde decontamination was determined by the number of positive spore strips and the incubation time at which a positive result was observed. Fewer positive strips after a longer incubation period indicated a higher level of decontamination. Titrations showed that less than ten residual viable spores produced visible bacterial growth in 18 hours. Results of spore strip monitoring after seven days incubation are shown in this report and are thus a most sensitive index of the effectiveness of the formaldehyde decontamination.

In recording the results of decontaminations of room spaces and chambers, more than 95% of decontaminations resulted in the inactivation of four or more of the five spore strips (Table 1). In intermediate cases, more than one test spore strip produced positive growth while others were negative. Although this indicated less stringent decontamination, a highly formaldehyde-resistant indicator organism had been used. Such results were interpreted by the laboratory as providing satisfactory decontamination as any small bioburden of animal pathogens of concern would have been inactivated during the process.

Formaldehyde for the decontamination of filter canisters was generated by boiling formalin solution, rather than by subliming paraformaldehyde. This method was chosen as a small volume of formalin solution could be injected into the decontamination rig safely and efficiently through a port and funnel over the heating pan. The volume of formalin used in each case was calculated to produce 11.5 g/cu. m of formaldehyde. The higher formaldehyde concentration is thought to

have been necessary in this application because the large surface area of the filters *in situ* may have adsorbed some of the formaldehyde and reduced the active concentration in the gaseous phase.

For filter canisters, only two spore strip controls were used for each decontamination because of limitations in placing additional strips in the (potentially contaminated) sealed canister. Of 604 experiments done, at least one spore strip was positive on 17% of occasions (Table 2). This success rate was slightly less than that achieved for the larger room spaces. It was noted, however, that spore strip growth was more likely to occur during the colder months. This was explained in terms of a possible decrease in relative humidity in these months as the supply air is heated only and not humidified as it enters the building. During winter, this represents a temperature rise, on average, from 10°C to 21°C with a concomitant fall in relative humidity. It became laboratory practice in winter, therefore, to pay particular attention to the calculation of the volume of water required to increase the relative humidity during decontaminations. Additional water during the colder months each year reduced the seasonal clustering of decontamination failures. The higher volumes of water were not used throughout the year to reduce the possibility of water vapor saturation and condensation leading to a reduction in effectiveness of the decontamination (Lach, 1990). Psychrometric charts were used to assess accurately the volume of water required to raise the humidity to any required value. Rule of thumb volumes based on experimental and operational experience for three ambient humidity ranges were routinely adopted.

When the decontamination data of Class II biological safety cabinets was analyzed, 7% and 11% of spore strip tests showed positive growth for either one strip or two (or more) strips, respectively. These results were consistent with those obtained for the two other types of decontaminations discussed. For decontaminations in both biological safety cabinets and filter canisters, the large surface area HEPA filters may cause an adsorption of formaldehyde and water from the gaseous phase, which would explain the lowered observed effectiveness of decontaminations in these units.

The conclusions derived from this work are that gaseous formaldehyde decontamination of laboratory spaces, surfaces, and equipment can be used routinely with an 81-92% confidence that *B. stearothermophilus* spores will be completely inactivated on all test spore strips, a high level of decontamination. The most likely reasons for incomplete inactivation in some cases are that temperature and/or relative humidity are below the optimal values and that this occurs more frequently during colder weather at this laboratory.

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