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# Effective Decontamination of Laboratory Animal Rooms with Vapour-phase ("Vaporized") Hydrogen Peroxide and Peracetic Acid

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***Summary***

The purpose of this study was to investigate the decontaminant effects of vapour-phase hydrogen peroxide (VHP) and peracetic acid (PAA) in laboratory animal rooms. Methodologically and microbiologically, both methods were evaluated as an alternative to traditional methods. In the VHP decontamination process, the cycle consisted of 4 phases (dehumidification, conditioning, decontamination and aeration). The residual vapour was catalytically decomposed into water and oxygen. The complete process of room decontamination with VHP took 14-15 hours.

In PAA decontamination, the dry fog system produced very fine droplets of disinfectant that were dispersed throughout the laboratory animal rooms. The overall decontamination process by PAA mist took 3 hours, which was much faster than VHP decontamination. In both methods, no corrosion appeared on the material surfaces. The results of chemical and biological indicators showed complete decontamination after exposure to VHP and PAA fumigation. In the airborne microbiological examinations, total colony counts for the surface and environmental microorganisms were minimal. There were no significant differences between VHP and PAA fumigation. In laboratory facilities, conventional decontaminating methods will be gradually converted into VHP or PAA decontamination. This experiment confirmed that the VHP method is suitable for the decontamination of a relatively limited space whereas the PAA method is applicable to the rapid decontamination of spacious laboratory animal rooms. In conclusion, decontamination with VHP and PAA holds great promise as an effective alternative to currently used formaldehyde fumigation.

*Abbreviations*

ACC: peracetic acid, BIs: biological indicators, CFU: colony-forming units, CIs: chemical indicators, EPA: Environmental Protection Agency, PDACP: potato dextrose agar with chloramphenicol, SCD: soybean casein digest, SCDLP: soybean casein digest with lecithin and polysorbate 80, and VHP: vapour-phase hydrogen peroxide.

***Introduction***

In the past, laboratory animal rooms and equipment that could not otherwise be treated have been decontaminated with formaldehyde fumigation. Traditional fumigation with formaldehyde-based agents is potentially hazardous for both the workers and the environment (*NIOSH, 1981; OSHA, 1991; 1993; 2002; Rutala et al., 2008*).

Recently, vapour-phase ("vaporized") hydrogen peroxide (VHP) and peracetic acid (PAA) methods have been used to decontaminate laboratory and medical equipment and pharmaceutical manufacturing facilities (*Klapes & Vesley, 1990; Working Party Report, 1998; Krause et al., 2001; Uebel & Nicholson, 2006*). VHP is an antimicrobial decon-

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taminant which inactivate bacterial spores on environmental surfaces in an enclosed area (*Klapes & Vesley, 1990*). It is used in commercial, institutional and industrial settings to decontaminate or sterilize sealed enclosures such as isolators, workstations, pass-through rooms, medical and diagnostic devices, and for other biological safety applications (*French et al., 2004; Tilton & Kauffman, 2004; Small & Deitrich, 2007*).

A cold sterilant based on peracetic acid and hydrogen peroxide is registered with the Environmental Protection Agency (EPA) for use as an easy-to-use fog to enhance existing cleaning and disinfection processes. Meanwhile PAA decontamination is currently widely used in pharmaceutical clean rooms.

There is an urgent need for an alternative to traditional methods in laboratory animal facilities. However, there is a paucity of experimental data applicable to VHP and/or PAA decontamination of laboratory animal rooms. The purpose of this study was to investigate the decontaminant effects of VHP and PAA mist in laboratory animal rooms. Methodologically and microbiologically, both methods were evaluated as an alternative to traditional methods.

## *Materials and Methods*

### *VHP decontamination*

The experiments were conducted in 4 air-conditioned laboratory animal rooms (room 1: 44.5 $m^3$; room 2: 50.0 $m^3$; room 3: 44.5 $m^3$; room 4: 50.0 $m^3$), including 4 stainless-steel racks and a desk. VHP fumigation was performed with a VHP M100 decontamination system (Santasalo & Steri-Pro Solution Co., Kobe, Japan).

The cycle consisted of 4 phases: 1. dehumidification, 2. conditioning, 3. decontamination and 4. aeration. 1: During dehumidification, the relative humidity was reduced to 10-30% by circulation of the air in a closed loop; 2: During conditioning, VHP was produced by vaporization of 35% aquarous hydrogen peroxide and was introduced into the recirculating air stream to achieve the desired VHP concentration rapidly; 3: The decontamination phase proceeded to the conditioning phase at a steady-state injection and recirculation flow rate to maintain the VHP concentration for the desired exposure time; 4: During the aeration phase, VHP was no longer introduced, and then the residual vapour was catalytically decomposed into water and oxygen by recirculation through the destroyer or by using the room ventilation system after decontamination. The VHP M100 microprocessor automatically monitored and/or controlled the process parameters during each cycle. Cycle parameters used for the exposure to VHP are listed in Table 1.

**Table 1.** Cycle parameters used for the exposure to VHP

| Parameters | Run |
|---|---|
| **Dehumidification** | |
| Air flow ($m^3$/l) | 34.0 |
| Absolute humidity (%) | 30 |
| Time (min) | 10 |
| **Conditioning** | |
| Air flow ($m^3$/l) | 30.0 |
| Injection rate (g/min) | 6.0-8.0 |
| Time (min) | 50 |
| **Decontamination** | |
| Air flow ($m^3$/l) | 30.0 |
| Injection rate (g/min) | 3.5-5.0 |
| Time (min) | 120 |
| **Aeration** | |
| Air flow ($m^3$/l) | 30.0 |
| Time (min) | 720 |

### *PAA-VHP decontamination*

The dry fog system produced very fine droplets (6.0 μm in diameter) of disinfectant that were dispersed throughout the laboratory animal rooms. PAA decontamination was conducted in 4 laboratory animal rooms as above. The disinfectant used for this system was a cold sterilant solution consisting of a stable mixture of peracetic acid (PAA) and hydrogen peroxide. The dry fog unit (OZMIC-CJ-1, Oz Sangyo Co. Ltd., Tokyo, Japan) was positioned on the floor near the center of the room. During the dry fog process, the humidity level of the room to be treated was first raised to 80%. Then, the dry fog



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solution was evenly and completely dispersed in the room. Following the diffusion procedure (1 hour) and the hold time (1 hour), it took 2 hours to reduce the disinfectant level to allow safe re-entry.

*Microbiological examinations*

Air temperature and humidity were monitored by a thermohydrometer (Model CT 485, Newport Electronics, Deckenpfronn, Germany). Draeger glass vials (No. 8101041, Draeger, Luebeck, Germany) were used to monitor hydrogen peroxide concentrations in the laboratory animal room and adjacent areas.

Decontamination validation was performed with chemical and biological indicators placed throughout the room. After the decontamination cycle, the chemical indicators (VHP indicator, Santasalo & Steri-Pro Solution Co., Kobe, Japan) were examined for colour changes indicative of the presence of VHP. The biological indicators (Steris Sporedex-VHP-Bioindicators, Santasalo & Steri-Pro Solution Co., Kobe, Japan, consisting of $5 \times 10^5$ spores of *Geobacillus stearothermophilus*) were recovered and incubated in growth media (CASA medium, Merck, Darmstadt, Germany) at 55°C for 7 days, to indicate the presence or absence of growth. In the airborne microbiological examinations, total colony counts of environmental microorganisms were determined with soybean casein digest agar (SCD, Nissui Pharmaceutical Co., Tokyo, Japan) and potato dextrose agar with chloramphenicol (PDACP, Nissui Pharmaceutical Co., Tokyo, Japan). Surface contamination was monitored by two kinds of contact plates: soybean casein digest agar with lecithin and polysorbate 80 (SCDLP, Nissui Pharmaceutical Co., Tokyo, Japan) and PDACP. All plates were incubated in the incubator at 37°C for up to 2 days or 25°C for up to 14 days. Results were recorded as total colony-forming units (CFU) of bacteria or fungal counts per plate.

*Statistical analysis*

Statistical analysis on the microbiological data was performed using the *Fisher*'s exact probability test. A value of $p < 0.05$ was used to indicate significance for all analyses.

***Results***

*VHP decontamination*

Throughout the decontamination processes, condensation of hydrogen peroxide was not observed in the room and/or on its components. No corrosion, cosmetic changes or residues subsequently appeared on the material surfaces. Adverse effects of VHP gas were not found in the various types of equipment.

The concentrations of hydrogen peroxide vapour were constantly monitored at two positions in the laboratory animal rooms. The result of VHP concentrations is shown in Figure 1. The VHP levels reached their maximum concentrations at 1-2 hours after beginning of this decontamination. During room fumigation with VHP, hydrogen peroxide concentrations were monitored from 200 p.p.m. to 300 p.p.m. but were below 1 p.p.m. 15 hours after beginning of the aeration process. The complete process of room decontamination with VHP took 14-15 hours with the aeration phase run overnight for safety.

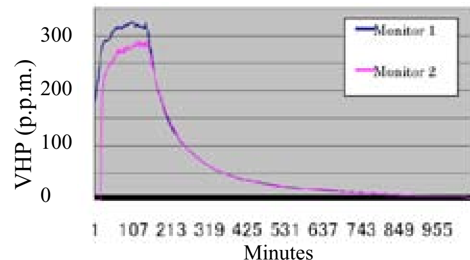


**Figure1.** The changes of VHP concentrations in the laboratory animal rooms.

*PAA decontamination*

The dry fog system allowed very fine droplets of PAA to be delivered rapidly to every corner of the room. These fine droplets effectively reached the material surfaces in the distant areas and thus avoided excessive condensation or corrosion of equip-



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ment. These droplets evaporated and their vapour penetrated normally inaccessible areas. No residues of PAA were detected above the acceptable ranges after the room ventilation.

*Biological examinations*
The results of CIs and BIs after exposure to both decontaminants are summarized in Table 2. CIs showed qualitative results to measure the distribution and concentration of hydrogen peroxide gas and PAA mist. In this study, all of CIs evenly changed from blue to grey in colour. These exposed CIs revealed that hydrogen peroxide gas and PAA mist reached the representative locations in sufficient quantity.
Culturing of BIs then demonstrated a complete inactivation of all spores of *Geobacillus stearothermophilus* due to a lack of spore growth after incubation in growth media.

**Table 2.** The results of chemical and biological indicators after decontamination procedures

| Indicators | VHP | PAA |
|---|---|---|
| Chemical indicators (CIs) | 0/16 | 0/12 |
| Biological indicators (BIs) | 0/16 | 0/12 |

In the excellent results from both the BIs and CIs strips, there were no differences between VHP and PAA decontamination. These positive results from all the CIs proved that VHP and PAA mist could reach all parts of the equipment and all areas of the room. BIs showed that both the decontaminants effectively inactivated spore-forming heat resistant organism (*Geobacillus stearothermophilus*). The overall decontamination process by PAA mist took 3 hours, which was much faster than VHP decontamination.
In the airborne microbiological examinations, only two SCD plates demonstrated bacterial growth (1 CFU) in the room treated with VHP (Table 3). No significant differences were found between VHP and PAA methods ($p = 0.157$). The adherence microorganism examinations showed slight growth (1 CFU) in only three SCDLP contact plates which were obtained from the rooms treated with PAA (Table 4). However, no significant differences were observed between VHP and PAA methods ($p = 0.205$). After two kinds of decontamination procedures, no noteworthy contaminants were found in the microbiological examinations. In microbiological disinfection, there were no significant differences between VHP and PAA fumigation.

**Table 3.** The results of airborne bacteria and fungi via the setting plate sampling

| Agar mediums | VHP | PAA |
|---|---|---|
| SCD | | |
| Room 1 | 1/4 | 0/6 |
| Room 2 | 0/4 | 0/4 |
| Room 3 | 0/1 | 0/5 |
| Room 4 | 1/4 | 0/4 |
| Total | 2/13 | 0/19 |
| PDACD | | |
| Room 1 | 0/4 | 0/6 |
| Room 2 | 0/4 | 0/4 |
| Room 3 | 0/1 | 0/5 |
| Room 4 | 0/4 | 0/4 |
| Total | 0/13 | 0/19 |

SCD: Soybean casein digest agar mediums
PDACD: Potato dextrose agar mediums with chloramphenicol

***Discussion***
VHP generators are used in the pharmaceutical manufacturing industry to sterilize isolators that are used for the aseptic processing of drugs and medical devices (*Lipman, 2007*). However, the utilization of VHP is limited to special biomedical research facilities because of the initial capital expense of the equipment (generating and dehumidifying equipment).
Several studies reported that the VHP process was highly effective against various microorganisms



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**Table 4.** The results of adherence bacteria and fungi after decontamination procedures

| Agar mediums | VHP | PAA |
|---|---|---|
| SCDLP | | |
| Room 1 | 0/10 | 1/16 |
| Room 2 | 0/10 | 1/10 |
| Room 3 | 0/5 | 0/15 |
| Room 4 | 0/9 | 1/9 |
| Total | 0/34 | 3/50 |
| PDACD | | |
| Room 1 | 0/10 | 0/16 |
| Room 2 | 0/10 | 0/10 |
| Room 3 | 0/5 | 0/15 |
| Room 4 | 0/9 | 0/9 |
| Total | 0/34 | 0/34 |

SCDLP: Soybean casein digest agar mediums with lecithin and polysorbate 80
PDACD: Potato dextrose agar mediums with chloramphenicIo

including bacteria, yeast, fungi, viruses, bacteria spores and prions (*Heckert et al., 1997; Kahnert et al., 2005*). In microorganisms, hydrogen peroxide works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA and other essential cell components. Although catalase can protect cells of microorganisms, VHP concentrations used for disinfection overcome this cell defence (*Rutala et al., 2008*). In this study, we confirmed that the VHP process was an excellent method for decontaminating both the laboratory animal rooms and their equipment. As reported in previous papers (*Krause et al., 2001, Kahnert et al., 2005*), VHP was compatible with laboratory animal rooms including experimental equipment such as racks and cages.

The VHP process, one needs to remove moisture from the space to be decontaminated prior to release of VHP. It was important to ensure that the VHP concentration should be kept below the condensation point; condensation (from the vapour to a liquid phase) of hydrogen peroxide can be damaging to surfaces and presents safety risks in room decontamination. VHP decontamination was initiated by preconditioning the air to remove water vapour. Hydrogen peroxide vapour was then introduced continuously and returned to the generator, where it was converted to oxygen and water vapour. In this study, VHP was active in the vapour phase and its effectiveness developed in the optimal environmental moisture.

PAA is dispersed as a spray, as the solution is sporicidal in the vapour phase as well as the liquid phase and thus kills microorganism suspended in the air and on surfaces that escape wetting (*Rutala et al., 2008*). A major advantage of PAA that is effective both in vapour and liquid phase. After PAA mist was sprayed at room temperature, PAA inactivated the most resistant bacteria and mould spores within 15 minutes. Optimal sporicidal activity of PAA in the vapour phase was empirically achieved at 80% relative humidity with no signs of corrosion. This method was particularly useful for decontaminating complex equipment that was difficult to clean manually. Our results showed that the PAA method could be applied in laboratory animal rooms to decontaminate working surfaces and the surface of equipment. Additionally, this method reduced the work of caretakers and lowered operation costs.

PAA is characterized by rapid action against all microorganisms. Although little is known about its mechanism of action, it is believed to function similarly to other oxidizing agents. PAA denatures proteins, disrupts the cell wall permeability and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites (*Rutala et al., 2008*). Notable advantages of PAA are as follows: 1. PAA lacks harmful decomposition products, 2. PAA enhances removal of organic material, 3. PAA remains effective in the presence of organic matter and 4. PAA leaves no residues (*Rutala et al., 2008*).

In both decontamination procedures, total colony counts for the surface and environmental microorganisms were kept to minimum CFU. The manufactures suggest the use of BIs (*Geobacillus stearothermophilus* spore strips) both at the time of installation and routinely to ensure effectiveness of the process. As long as chemical monitoring strips de-



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tect that the active ingredient is > 1500 p.p.m., CIs are also available for routine use as an additional process control (*Rutala et al., 2008*). The results of CIs and BIs showed complete decontamination after exposure to VHP and PAA fumigation. These findings in PAA decontamination were comparable to those achieved using VHP decontamination.

PAA is not considered a carcinogen by the Environmental Protection Agency, Occupational Safety and National Toxicology Program, and is also not genotoxic or mutagenic (*Rahija, 2007*).

Traditional fumigation with formaldehyde is effective in disinfecting animal rooms. However, formaldehyde fumigation is toxic and harmful for caretakers and the environment (*NIOSH, 1981; OSHA, 1991; 1993; 2002; Rutala et al., 2008*). This method has currently suffered the effects of strict government regulations on the use of formaldehyde vapour. In laboratory facilities, conventional decontaminating methods (formaldehyde or other manual wipe-down procedures) will be gradually converted into VHP or PAA decontamination. The decomposition products of PAA are acetic acid, hydrogen peroxide, oxygen and water, and then the hydrogen peroxide breaks down into oxygen and water. PAA decontamination was considered to be a safe and effective alternative to formaldehyde fumigation. From this experiment, I confirmed that the VHP method was suitable for the decontamination of relatively limited space. In contrast, the PAA method was applicable to the rapid decontamination of spacious laboratory animal rooms. In conclusion, decontamination with VHP and PAA, either separately or together depending on the space to be treated, holds great promise as an effective alternative to currently used formaldehyde fumigation.

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