Establishment of a Vaporous Hydrogen Peroxide Bio-Decontamination Capability

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ABSTRACT

Vaporous hydrogen peroxide (VHP) has previously been demonstrated to be effective against biological agents and to some extent chemical agents. As part of the current HPPD work program on chemical and biological decontamination a VHP decontamination system was acquired. Here we describe the commissioning of the VHP unit which involved setting up a small scale testing capability, the sterilisation of a PC3 laboratory and conducting a field trial to decontaminate equipment in a tent. In each case positive results were obtained regarding the distribution of the vaporous decontaminant and its efficacy as a biological decontaminant.

RELEASE LIMITATION

Approved for public release
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Executive Summary

Vaporous hydrogen peroxide (VHP) is an effective bio-decontaminant and has been used to sterilise laboratories for over a decade. This technology was used in the building remediation efforts that followed the 2001 anthrax attacks in the United States. Since that time, researchers at the Edgewood Chemical Biological Center (ECBC) and Steris Corp. have continued to develop this technology for large scale decontamination of buildings, aircraft interiors and sensitive equipment. Following positive results, a VHP decontamination system was acquired by HPPD as part of the current work program on chemical and biological decontamination. This system vaporises hydrogen peroxide solution to generate a dry decontaminant in the gas phase. VHP has previously been demonstrated to be effective against biological agents, including \textit{Bacillus Anthracis}, as well as a number of chemical agents under certain circumstances.

Here we describe the commissioning of the VHP system. This involved establishing a small scale testing capability, the sterilisation of a physical containment 3 (PC3) laboratory at HPPD and a limited objective field trial to decontaminate equipment inside a tent using VHP. In each case positive results were obtained regarding the distribution of the vaporous decontaminant and its efficacy as a biological decontaminant. As part of the current program in HPPD this system will be used to better understand the processes involved in the decontamination method. In addition, the capability described will allow for the evaluation of the VHP technology for the decontamination of sensitive equipment and other material relevant to Australian Defence.
Dr Andrew McAnoy
Human Protection and Performance Division

Dr Andrew McAnoy graduated with a PhD from the University of Adelaide in 2003 for his research into the chemistry of molecular species of interstellar interest. In 2004, he commenced a Postdoctoral Fellowship at the University of Colorado at Denver and Health Sciences Center. There he utilised mass spectrometry to investigate the biochemical pathways involved in lipid metabolism. Andrew joined DSTO in August 2005 and has focussed on the potential use of vaporous decontamination methods for chemical and biological contamination control.

Michelle Sait
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Michelle Sait graduated from the University of Melbourne in 2000 with a B.Sc (Hons). She is currently finalising her PhD thesis which was performed in the department of Microbiology and Immunology, developing molecular tools to enable the isolation of novel soil bacteria. Her work at DSTO focuses on the development of assays for the molecular detection of microorganisms.

Sue Pantelidis
Human Protection and Performance Division

Sue Pantelidis commenced at DSTO in 1989 after graduating from Latrobe University with a Bachelor of Science degree in 1987, majoring in chemistry and applied mathematics. Initially she provided support to research into the fundamental chemical and physical properties of chemical warfare agents and related compounds by kinetic/mechanistic studies and various analytical techniques (NMR, GC). Since then she has worked in a variety of areas including: (a) computer simulation of vapour dispersion for early warning to vapour hazards, (b) preparation of antibodies and development of assays for the detection of ricin, and (c) evaluation of high affinity reagents and biosensing technologies for detection of biological warfare agents.
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1. Introduction

The intentional release of sarin nerve gas in a Tokyo subway system (1995) and the release of *Bacillus anthracis* (anthrax) spores through the United States mail system (2001) demonstrated the vulnerability of critical infrastructure to chemical and biological attack. These incidents also highlight the importance for containment and remediation of contaminated sites as rapidly and effectively as possible to reduce the impact to public health and to ensure safe and timely resumption of normal activities at the affected site. The requirement for rapid and effective response to a chemical or biological (CB) incident is also vital for Defence sites and equipment, particularly when there is a requirement to maintain a high degree of operational readiness.

Vaporous decontamination involves the application of a decontaminant in the vapour phase for the decontamination of enclosed spaces. In vapour form these decontaminants have the potential to decontaminate hard-to-reach and complex surfaces including; heating, ventilating and air conditioning (HVAC) systems, internal components of electronic equipment, as well as cracks in floors, walls and other surfaces which may be contaminated by aerosolised biological agents or agent vapours. A number of vaporous sterilants can be used to inactivate biological agents such as *B. anthracis* and these include beta-propiolactone, chlorine dioxide, ethylene oxide, propylene oxide, ozone, methyl bromide (MeBr), formaldehyde and hydrogen peroxide [1]. The state of these technologies has previously been discussed as part of a DSTO report [2]. Recommendations from the report resulted in the establishment of the current vaporous decontamination work program for the potential remediation of buildings, vehicles and sensitive equipment.

Central to the current DSTO program is vaporous hydrogen peroxide (VHP) which is an effective decontaminant against many microorganisms and has been used to sterilise laboratories and clean rooms for over a decade [3,4]. Edgewood Chemical Biological Center (ECBC) and Steris Corporation have adapted VHP for Defence purposes, with its effectiveness against anthrax spores demonstrated during the building remediation efforts which followed the 2001 anthrax attacks in the United States [5]. Since then VHP technology has continued to be developed for large scale decontamination of buildings, aircraft and sensitive equipment [6 - 11]. VHP has also been shown to be effective against chemical agents, an effect which has been observed to increase with the addition of ammonia gas. The resultant decontaminant is known as modified VHP (mVHP) and has potential as a broad ranging CB decontaminant to fulfil a number of decontamination requirements. Modified VHP is now being optimised by ECBC and is considered to be the lead technology for acquisition under the Joint Material Decontamination System (JMDs) in the United States.

As part of DSTO’s work program in decontamination a customised Steris 1000ED VHP/mVHP bio-decontamination system has been acquired to carry out controlled experimental studies to investigate the decontamination procedure. This system allows for the injection and monitoring of ammonia in addition to hydrogen peroxide during the decontamination process. The unit has a wide volume range that allows for the scaling up from small scale testing in a 1 m$^3$ enclosure to larger scales up to 210 m$^3$. A sensor array, with hydrogen peroxide (0 - 2000 ppm), ammonia (0 – 100 ppm), temperature and humidity sensors, was also provided to allow the system to measure experimental variables and enable feedback injection control of the decontaminant.
Herein, we describe the small scale VHP bio-decontamination testing and large scale VHP laboratory sterilisation capabilities of the acquired VHP system. Finally, results of a limited field trial involving VHP decontamination of equipment in a 75 m$^3$ tent will be discussed.

2. Vaporous Decontamination

2.1 Hydrogen Peroxide as a Vaporous Decontaminant

2.1.1 Overview

Vaporous hydrogen peroxide (VHP) is an oxidizing agent effective against many microorganisms including bacterial spores [1 - 5]. The VHP technology has been used for over a decade to sterilise pharmaceutical processing equipment and clean rooms. It is commercially mature and has demonstrated scalability to large spaces up to 5,660 m$^3$ [5 - 8]. VHP is generated by vaporising a solution of 30 - 35 % hydrogen peroxide. Since hydrogen peroxide catalytically decomposes to water and oxygen, VHP has the benefit of being effective against microorganisms while generating no harmful by-products or waste.

The UK-based Bioquell Corporation has commercialized a decontamination system using hydrogen peroxide vapour (HPV*) for bio-safety cabinet and room bio-decontamination [12]. The company has recently launched a room bio-decontamination service in the UK to combat hospital acquired infections [13]. The room decontamination process is rapid, with a typical hospital ward taking 12 hours, and is reported to not damage any of the sensitive hospital equipment. Recent trials conducted by Bioquell in association with hospitals in the UK have shown that while regular cleaning was poorly effective against high levels of (environmental) methicillin-resistant Staphylococcus aureus (MRSA), HPV was dramatically effective [14,15]. The Bioquell technology has also been tested by the EPA in the United States under the Environmental Technology Verification (ETV) program for application in biological agent decontamination [16]. The efficiency against B. anthracis was demonstrated by a log reduction in spores of 6.9 (or better) on non-porous surfaces and 3.0 (or better) on porous surfaces. HPV has also been shown to be effective against numerous other microorganisms and a recent report indicates that HPV may also be effective against prions [17].

After the anthrax attacks in the United States, VHP was one of the decontaminants permitted by the EPA to be used as a fumigant in the remediation effort and was successfully used to fumigate anthrax contaminated buildings [5]. Since then, there has been considerable interest in further developing VHP processes for the large scale decontamination of fixed sites and sensitive equipment after an attack involving a CB agent [5 - 11].

There has been much debate as to the exact mechanism involved in the decontamination of surfaces by vaporous hydrogen peroxide. The VHP advocates suggest a dry gas process is involved and any condensation is to be avoided, while the HPV supporters suggest that micro-condensation actually increases decontamination efficacy. These distinct mechanisms form the basis of two commercial

* HPV and VHP are both hydrogen peroxide in the vapour phase. HPV is used in this instance to distinguish the Bioquell micro-condensation process from the Steris dry gas process.
decontamination systems produced by Steris and Bioquell respectively [13,18]. An independent report compared the two systems and determined that both can be validated and that these differences do not affect the effectiveness of either unit to decontaminate [19]. Regardless of which decontamination process is used, scalability is apparently relatively easy. For example, the room decontamination system of Bioquell is modular and simply requires additional modules for larger spaces and/or sectioning of the large spaces into smaller spaces [13]. Additionally, ECBC and Steris have developed a self-contained VHP system on a trailer for larger decontamination requirements [6,8].

2.1.2 Decontamination of Buildings, Large Platforms and Sensitive Equipment

Recently, the activation of VHP toward chemical agents including GD, VX and HD has been investigated by ECBC where it was demonstrated that modifying the VHP decontamination process by adding low levels of ammonia gas increases efficacy against chemical agents [6 - 11].

Large scale testing of mVHP has been conducted in a building at Aberdeen Proving Grounds and in a C-141 aircraft at Davis-Monthan Airforce Base [6,8]. In the building test environment, a 24 hr mVHP treatment cycle, with 250 ppm of VHP and 20 ppm of ammonia, effectively reduced contact and vapour hazards of GD and VX to the limits of detection. While hazards of HD were reduced, trace amounts of HD vapour were detected, especially from porous surfaces. The aircraft test involved the use of a self-contained mVHP trailer unit which was used to obtain 125, 250 and 450 ppm levels of mVHP in the 14,000 m³ aircraft interior for a 5 hour treatment cycle. In the ECBC tests, computational fluid dynamic (CFD) modelling was used to model vapour distribution in the space to be decontaminated to assist in setup of the decontamination system and placement of sensors [5].

A prototype sensitive equipment decontamination (SED) system based on mVHP technology (Figure 1) was evaluated for operational utility at the Decontamination Limited Objective Experiment (LOE) in June 2005 at Tyndal AFB in the United States. This experiment indicated that mVHP has the potential for decontamination of sensitive equipment. Since that time ECBC and Steris have continued to develop the mVHP process using the prototype SED system [10]. Higher concentrations of hydrogen peroxide and ammonia have been found to increase the rate of decontamination and its efficiency. The prototype SED box using mVHP was shown to decontaminate chemical agents (HD, GD, TGD, VX) on various materials. Results and requirements varied based on the agent and material contaminated, but overall mVHP was shown to be effective and significantly reduced contamination levels. In many cases residual concentrations were close to, or below, operational objectives. Studies have also shown that for concentrations of 500 ppm of hydrogen peroxide and 30 ppm of ammonia, mVHP can achieve a 6-log reduction in *B. anthracis* Ames spores within 5 minutes on operationally relevant materials [11]. Under the same conditions spores of *G. stearothermophilus*, a common biological indicator (BI), required 30 – 60 mins. It was shown that *G. stearothermophilus* consistently required 15 times longer to achieve the same level of spore reduction as *B. anthracis*. It has been suggested that the SED prototype may be able to decontaminate 300 items within 2 hours, possibly less.
Figure 1  Prototype VHP sensitive equipment decontamination system based on a 463L pallet configuration developed in the USA by Steris and ECBC. Also shown is a scaled down version using a MM8 box as the enclosure. (Photographs courtesy of Steris Corp. and reproduced with permission).

Figure 2  Schematic representation of the VHP process showing the involvement and sequence of the major components involved.
2.1.3 The VHP Process

The VHP process involves controlling the injection and vaporisation of a 35 % hydrogen peroxide solution to maintain a sufficient hydrogen peroxide concentration in the gas phase. A schematic diagram of the main components of the VHP system and the direction of air/vapour flow during the VHP cycle is shown in Figure 2. The enclosure is connected to the unit via 1.25 inch PVC tubing with Cam-lock fittings. All remaining components are self contained within the unit and controlled by the system.

The VHP process has four phases: dehumidification, conditioning, decontamination, and aeration.

**Dehumidification**
Prior to the injection of hydrogen peroxide, dry heated air can be circulated through the enclosure to reduce the relative humidity and avoid condensation of hydrogen peroxide and water during the conditioning and decontamination phases. Sensors inside the enclosure monitor the temperature and humidity to ensure preset conditions are achieved. Dehumidification allows higher concentrations of hydrogen peroxide to be maintained in the enclosure without condensation and thereby minimises the potential for surface damage. Condensation is dependent on the initial temperature, humidity and the target concentration for the process.

**Conditioning**
In this phase hydrogen peroxide is vaporised and introduced into the enclosure. Typically high injection rates are used to rapidly raise the concentration to the target concentrations as measured by hydrogen peroxide and ammonia sensors inside the enclosure. After reaching the target concentrations the injection rates may be lowered to maintain the concentration levels.

**Decontamination**
During this phase injection of hydrogen peroxide continues at a sufficient rate to maintain the target condition for a predetermined amount of time. Effective decontamination is dependent on both concentration and time, with exposure measured as concentration-time (CT) in ppm-hrs. Therefore, higher concentrations of hydrogen peroxide require less time to achieve the desired CT than lower concentrations of hydrogen peroxide, as illustrated in Figure 3. It should be noted that localised variations in hydrogen peroxide concentration can occur due to uneven distribution of the vapour, adsorption and/or catalytic decomposition of hydrogen peroxide at the surface. Therefore, the measured CT may not always represent the actual CT experienced at all surface locations and so the target CT should always incorporate an adequate safety margin.

**Aeration**
Following sufficient decontamination time the system stops injection of hydrogen peroxide and circulates only dry air into the enclosure. The dried air displaces the remaining hydrogen peroxide vapour in the enclosure which is passed through the catalytic converter where it is converted to water and oxygen. Dried air continues to be passed through the enclosure until the decontaminant is removed.
Figure 3  Simulated plot of hydrogen peroxide concentration and cumulative concentration-time (CT) for the VHP process demonstrating the effect of varying concentration and time on CT. Each simulated plot assumes ideal conditions requiring no dehumidification and consists of $\frac{1}{2}$ hr conditioning followed by decontamination for (a) 2 hrs at 270 ppm and (b) 1 hr at 430 ppm.

2.2 Commissioning of the Small Scale VHP Testing Capability

2.2.1 Experimental Setup

A small volume vapour enclosure (1 m$^3$) was designed and constructed specifically for decontamination experiments using VHP. The size of the enclosure had to take into account the preference for a small volume for easier handling as well as the lower volume limit of the 1000ED system. The volume range for the 1000ED system is approximately 0.3 - 210 m$^3$. It was decided that a 1 m$^3$ box would be suitable as this would avoid potential complications associated with over
pressurisation of enclosures at the lower end of the volume range. The chamber was constructed using ½ inch Perspex. This allowed for flexibility in the positioning of various access holes for the vapour inlet and outlet as well for any wiring required for the sensor array and other probes, fans or electronic equipment as needed. A small access door was included which swings upward and can be held open to allow for equipment to be setup inside the enclosure.

The enclosure needed to be placed inside a specific fume hood for safety reasons. The fume hood has a volume of around 3.5 m³ and so can easily accommodate a 1 m³ vapour enclosure. However, the sash opening height is only 750 mm and so a fixed enclosure with sufficient height to easily slide into the fume hood would need to occupy the majority of the bench space inside the fume hood and would limit the experimental utility of the capability. The solution involved construction of an enclosure in two sections, each 1000 x 1000 x 500 mm. The sections were placed individually inside the fume hood separately and then joined together to form a cube with volume of 1 m³. Adequate seals and clips were fitted to prevent gas leaks during the decontamination process. The door was found to leak when the enclosure was slightly pressurised and as an interim measure heavy duty tape was used to seal the door. The complete small scale testing capability with the VHP system connected to the 1 m³ Perspex enclosure and sensor array is shown in Figure 4.

Figure 4  VHP small scale setup showing VHP system, Perspex 1 m³ enclosure and sensor array.

2.2.2 Results and Discussion

The small scale VHP testing capability as shown in Figure 4 allowed hydrogen peroxide concentrations above 1000 ppm to be easily achieved using modest injection rates as shown in Table 1. The maximum airflow of 35 m³/hr was used and corresponds to one volume exchange approximately every 2 mins. The feedback control using the real time data supplied by the sensor array was also
evaluated using this setup and the system was able to maintain hydrogen peroxide concentrations at the tested levels of 250 and 500 ppm.

<table>
<thead>
<tr>
<th>Injection Rate (g/min)</th>
<th>Hydrogen Peroxide (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>360</td>
</tr>
<tr>
<td>2.0</td>
<td>630</td>
</tr>
<tr>
<td>3.0</td>
<td>920</td>
</tr>
<tr>
<td>4.0</td>
<td>1170</td>
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<tr>
<td>5.0</td>
<td>1410</td>
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</table>

The upper limit of hydrogen peroxide concentration which could be achieved in the small enclosure while maintaining dry conditions was tested by slowly increasing the hydrogen peroxide concentration until condensation occurred. In this instance the walls of the clear enclosure began to fog when the hydrogen peroxide concentration reached 1800 ppm. The measured temperature inside the enclosure at this point was 34 °C with 28 % relative humidity, however the walls may have been at a lower temperature due to the cooling effect of external air movement within the fume hood. In any case, the measured hydrogen peroxide concentration was well above any currently used or anticipated decontamination concentrations of hydrogen peroxide. Therefore, this demonstrates that for operationally relevant concentrations of hydrogen peroxide (i.e. < 1000 ppm) the decontamination process will remain under dry conditions throughout experiments using the described small scale setup.

The distribution of the vapour throughout the enclosure was tested by using 5 chemical indicator (CI) strips. These CI strips change colour on contact with hydrogen peroxide and were placed on the sensor array in the middle of the enclosure, the ceiling and various locations on the walls, including the front corner below the injection port. The airflow was set to 35 m$^3$/hr for the experiment and the CI strips changed colour at approximately the same time and to the same extent. In addition, 5 biological indicator (BI) strips (G. stearothermophilus) were co-located with the CI strips inside the enclosure and exposed to 800 ppm of hydrogen peroxide for 1 hr. The BI strips that were exposed to vaporous hydrogen peroxide and a positive control BI strip were transferred aseptically to 10 ml Trypticase soya broth (TSB) and incubated at 55 °C for 7 days. The broth containing the positive control BI strip exhibited turbid growth in less than 24 hrs. The 5 TSBs containing the BI strips exposed to hydrogen peroxide in the small enclosure did not exhibit bacterial growth after 7 days incubation. The results of the CI and BI strips suggest that the vapour was dispersed throughout the enclosure at sufficient concentrations for thorough bio-decontamination. However, it should be noted this is not an exhaustive examination of the vapour dispersion and that it may be dramatically altered when additional objects are placed inside the enclosure and so internal fans are recommended to assist vapour distribution. Further insight could also be obtained using computational fluid dynamics to model the vapour distribution within the enclosure.
2.3 Laboratory Sterilisation using VHP

2.3.1 Experiment Setup

The acquired decontamination unit is based on Steris Corp’s commercial VHP unit which is designed for room bio-decontamination using VHP. This specification was tested on HPPD’s PC3 laboratory as part of the commissioning of the decontamination system. The volume of the laboratory is approximately 150 m³, well below the upper limits of the VHP system and therefore provided an appropriate assessment of the room bio-decontamination capability of VHP system.

A simplified layout of the laboratory sterilisation process is shown in Figure 5. The extent of VHP distribution and efficacy of the process was tested using CI strips and BI strips (G. stearothermophilus) from two different suppliers, Steris Corp. (Mentor OH USA) and Consulchem Pty Ltd (Scoresby VIC Australia). These were positioned in 10 different locations around the laboratory, including under benches, inside a bio-safety cabinet and at ceiling height. The HVAC system was isolated and operated in recirculation only mode to assist distribution of the vaporous decontaminant. In addition, desktop fans were placed around the room and faced in the direction as indicated in Figure 5 in an attempt to rotate the air/vapour around the lab to evenly distribute the decontaminant.

The VHP unit was placed inside the lab with the outlet positioned away from the inlet, and pointed in the same direction as the fans, to ensure that freshly generated VHP did not flow directly to the inlet and bypass other areas of the laboratory. The sensor array was placed on top of a refrigerator at the inlet side of the VHP unit and for convenience directed toward a viewing window so that the concentration measured could be readily monitored external to the laboratory and ante-room. The control unit was removed from the unit* and reconnected via an extension lead so that the unit could be monitored and controlled remotely from within the ante-room. The door to the laboratory was sealed around the controller lead with heavy duty tape.

* Removal of the control unit is not for routine operations and was conducted with the assistance of the engineers from the manufacturer. An additional controller is recommended for routine use.
2.3.2 Results and Discussion

The temperature inside the laboratory was initially 25 °C with 37 % relative humidity and so it was unnecessary to dehumidify the laboratory. The process was started in the conditioning phase with the system in feedback control and set to achieve 300 ppm hydrogen peroxide as measured by the sensor array. The conditioning time was set to 30 minutes and the decontamination phase set to maintain the hydrogen peroxide concentration at 300 ppm for 2 ½ hrs. However, the system was unable to determine an appropriate injection level for the conditioning phase and injected less than 2.5 g/min of hydrogen peroxide for 35 min, which resulted in a hydrogen peroxide concentration inside the laboratory of only 60 ppm. At this time the system automatically increased the injection rate to 10 g/min and then 25 g/min which resulted in a system alarm which aborted the process and advanced the system to aeration. The process was manually restarted from the ante-room with the feedback control disabled. The process recommenced at the conditioning phase and injected hydrogen peroxide at a rate of 12 g/min for 1 hr. This achieved a hydrogen peroxide concentration of around 200 ppm in the laboratory at the end of the conditioning cycle.

On advancement to the decontamination phase the system tried to refill the hydrogen peroxide reservoir but timed-out and resulted in an alarm which triggered a phase advance to the aeration phase. As the process was unattended, 40 mins passed before the falling hydrogen peroxide concentration was noticed. The aeration phase was manually aborted and the decontamination cycle recommenced for a further 1 hr 40 min with a hydrogen peroxide injection rate of 12 g/min. After that time the final aeration phase commenced and an hour later the HVAC system was turned on to assist the final aeration of the laboratory overnight.
The concentration and cumulative concentration-time (CT) are plotted against time in Figure 6. The technical difficulties discussed above directly affected the measured hydrogen peroxide concentration. The extended conditioning phase and drop in hydrogen peroxide levels are clearly shown in the concentration curve (shown in red). Despite the fluctuating hydrogen peroxide concentration the cumulative CT curve (shown in blue) indicated that overall a significant CT value of around 800 ppm-hr was achieved. Given that conservative estimates for 6 log kill are CT values of 600 - 800 ppm-hr this indicated that the application of VHP for laboratory sterilisation was successful in this instance. The validation of the process was independently assessed based on the results from the CI and BI strips which were positioned throughout the laboratory during the process. The CI strips all showed positive response to hydrogen peroxide exposure indicating good distribution of the decontaminant throughout the laboratory. The 20 BI strips that were exposed to VHP (10 locations) and a positive control BI strip from each manufacturer were transferred aseptically to 10ml Trypticase soya broth (TSB) and incubated at 55 °C for 7 days. The broth containing the positive control BI strips exhibited turbid growth in less than 24 hours. The 20 TSBs containing the BI strips exposed to VHP did not exhibit bacterial growth after 7 days incubation.

In summary, the sterilisation of the PC3 laboratory was achieved using vaporous hydrogen peroxide. Despite the technical problems the unoptimised process was able to achieve a CT of around 800 ppm-h for VHP exposure, with the laboratory reaching a dose of 600 ppm-h after 4 hours. Based on these results it is anticipated that a CT of 600 ppm-hr for VHP exposure could be achieved within 3 hrs using a hydrogen peroxide injection rate of 12 g/ min. Furthermore, a CT of 800 ppm-hr would be expected to be achieved in less than 4 hrs.

![Figure 6](image)

*Figure 6* Results from the VHP sterilisation of the PC3 laboratory at HPPD showing the measured hydrogen peroxide concentration (ppm) in red, and the cumulative concentration-time (CT, ppm-hr) in blue.
2.4 Equipment decon in the field using VHP

2.4.1 Experimental Setup

A limited field trial was conducted using the VHP system at a remote location. The scenario of the trial was that of a biological contamination incident requiring decontamination of contaminated items inside an inflatable PVC tent with internal volume of 75 m$^3$. The objective was to verify that sufficient levels of VHP could be generated inside a tent in uncontrollable external conditions.

The tent layout with the locations of the VHP system, sensor array, tables and equipment is shown in Figure 7. Pedestal fans were placed on both sides of the tent and positioned in opposite directions to assist even distribution of the vaporous decontaminant. The sensor array was placed to measure hydrogen peroxide concentrations toward the return air end of the air cycle. Assuming thorough vapour distribution and a cyclic air movement around the tent, the concentrations measured should represent a lower limit. CI and BI strips were placed at various locations to determine the extent of VHP distribution and were used to determine success of the process.

Prior to decontamination the tent was sealed as much as possible. The tent was set up in a well ventilated area and therefore any leakage would be diluted in the ambient atmosphere. Local conditions in the tent prior to commencement of the decontamination were 20.4 °C with a relative humidity of 48 %. Based on previous results it was estimated that hydrogen peroxide concentrations above 200 ppm could be achieved within 30 min. Due to the modest target concentrations of hydrogen peroxide for this trial the VHP unit bypassed the dehumidification phase and was started in the decontamination phase for 100 min with an injection rate of 10 g/min. The experiment was left unattended with the aeration phase set to follow the decontamination phase and run for 6 hours to reduce the VHP concentration. *

Figure 7 Layout of 75 m$^3$ tent showing location of VHP system, sensor array, tables, equipment and pedestal fans.

* It should also be noted that if personnel remained in attendance during the VHP exposure then handheld H$_2$O$_2$ sensors would have been required to ensure a safe distance could be maintained around the tent.
2.4.2 Results and Discussion

The hydrogen peroxide concentration and CT observed during the limited field trial are given in Figure 8. The concentration of hydrogen peroxide steadily increased during the first 50 min of the decontamination phase reaching 200 ppm within 30 min. The concentration then stabilised to between 260 and 275 ppm for the remaining 50 min of the decontamination phase. At the end of the decontamination phase the concentration was measured at 274 ppm and the cumulative CT determined to be 386 ppm-hr. The aeration phase then commenced and continued unattended for 6 hours. At this point the hydrogen peroxide concentration was reduced to around 40 ppm and the system shut down. The tent was left overnight and the hydrogen peroxide concentration measured at 19.5 hrs was 5 ppm. As this was above the exposure limits, the tent doors and windows were fully opened and the tent was left to aerate to remove residual hydrogen peroxide.

In this instance, the aeration phase did not reduce the hydrogen peroxide concentration as readily as predicted due to off-gassing of absorbed hydrogen peroxide. The hydrogen peroxide concentrations measured through the 8 hrs of the experiment are plotted against time in Figure 8. After the injection of hydrogen peroxide stopped at 1hr 40 min, the system began to catalytically breakdown hydrogen peroxide inside the tent and the concentration rapidly decreased from 274 to 222 ppm within 30 min. However, as hydrogen peroxide levels decreased due to the catalytic breakdown process, off-gassing of absorbed hydrogen peroxide competed to increase levels as indicated by the shoulder to the concentration curve from 2 – 4 hrs. As off-gassing is dependent on the amount and type of material present this demonstrates the need to monitor the aeration process. In this case continued aeration for an additional 6 hrs would have further assisted the reduction of hydrogen peroxide to safe levels (< 1 ppm). Alternatively, supplementary aeration methods could be provided to assist breakdown of the hydrogen peroxide. This could include a simple system consisting of a fan connected to a standard catalytic converter. Such a system could then be switched on at the beginning of the aeration cycle to pass hydrogen peroxide over the active surface of the catalyst and thereby increase the rate of decomposition of hydrogen peroxide and decrease the aeration time.
2.5 Summary and Conclusions

A VHP capability has been established to support the current work program on decontamination of chemical and biological warfare agents. A purpose-built enclosure has been constructed to allow for testing within the laboratory environment and the ability of the VHP system to maintain levels of up to 1000 ppm of hydrogen peroxide inside the enclosure was demonstrated. It has been shown that the VHP system sterilised HPPD’s PC3 laboratory. Based on the results obtained it is anticipated that, with further optimisation of process variables, the sterilisation of the PC3 laboratory should be achievable in less than 4 hrs. Finally, the VHP unit was also used in a limited field trial to decontaminate equipment inside a tent. In each case positive results were obtained regarding the distribution of the vaporous decontaminant and its efficacy as a biological decontaminant.

Overall, the results described here using the acquired VHP system to decontaminate enclosures with volumes of 1 m$^3$, 75 m$^3$ and 150 m$^3$, highlight the current HPPD capability to evaluate VHP technology for the decontamination of sensitive equipment and material relevant to Australian Defence.

2.6 Acknowledgements

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3. References


13. Additional information available from company website, URL – www.bioquell.com


## Title
Establishment of a Vaporous Hydrogen Peroxide Bio-Decontamination Capability

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- Abstract: (U)

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### Abstract
Vaporous hydrogen peroxide (VHP) has previously been demonstrated to be effective against biological agents and to some extent chemical agents. As part of the current HPPD work program on chemical and biological decontamination a VHP decontamination system was acquired. Here we describe the commissioning of the VHP unit which involved setting up a small scale testing capability, the sterilisation of a PC3 laboratory and conducting a field trial to decontaminate equipment in a tent. In each case positive results were obtained regarding the distribution of the vaporous decontaminant and its efficacy as a biological decontaminant.