

Certification of Vapor Phase Hydrogen Peroxide Sterilization Process for Spacecraft Application

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ABSTRACT

In order to meet the National Aeronautics and Space Administration (NASA) planetary protection microbial reduction requirements for all Mars in-situ life detection and sample return missions, entire planetary spacecraft (including planetary entry probe and planetary landing capsules) may have to be exposed to a qualified sterilization process. Presently, dry heat is the only NASA approved sterilization technique available for spacecraft application. However, with the increasing use of various man-made materials, highly sophisticated electronic circuit boards, and sensors in a modern spacecraft, compatibility issues may render this process unacceptable to design engineers and thus impractical to achieve terminal sterilization of entire spacecraft. An alternative vapor phase hydrogen peroxide sterilization process, which is currently used in various industries, has been selected for further consideration. This paper describes the selection process and research activities JPL is planning to conduct for certification of hydrogen peroxide as a NASA approved technique for sterilization of various spacecraft parts/components and entire modern spacecraft.

INTRODUCTION

International agreements and the protection of the scientific integrity of information gathered by unmanned spacecraft venturing to other planets of biological interest, such as Mars, demand that such spacecraft are biologically clean^(1,2). Currently, dry heat is the only approved method for sterilization of an entire spacecraft or the component parts. Dry heat microbial reduction at 125°C for four hours (or 110°C for 47 hours) is still a valuable Planetary Protection tool for components which can readily withstand the elevated

temperature. However, many components of a modern spacecraft are stressed beyond design limits by such elevated temperatures and redesigning the hardware with less temperature sensitive materials would require extensive reworking of the components and associated testing and requalification of flight hardware. Thus, alternative techniques for sterilizing the surface of components or even of an entire spacecraft are being considered. Two such techniques selected for study are vapor phase hydrogen peroxide⁽³⁾ and chlorine dioxide^(4,5). These techniques have been used widely in the bio-medical community to sterilize such items as implantable cardiac pacemakers, catheters, and many other medical devices^(6,7). These relatively low temperature techniques also have the advantage of ease of disposal of chemical by-products after completion of the sterilization cycle.

In order to have any process certified as a NASA approved sterilization technique, the following technical issues need to be addressed:

1. Development of a three dimensional model to predict dead space and sterilant concentration profile in a fairly large sterilization chamber (large enough to accommodate a spacecraft) for a selected sterilant injection mode and sterilization cycle.
2. Selection of a standard organism, known to be resistant to the sterilization process.
3. Identification of microorganisms (including hardy ones) normally found in spacecraft assembly facilities.
4. Experimental demonstration of the process effectiveness in killing hardy microbes deposited on

exposed and hard to reach surfaces at a specified sterilization temperature and sterilant concentration for a specified time.

5. Experimental demonstration of process effectiveness in killing microbes deposited on various types of surfaces such as metallic, composite, polymeric, etc.
6. Establishment of complete sterilization specifications for application to a spacecraft.

This paper discusses these technical issues.

VAPOR PHASE STERILIZATION TECHNIQUES

Ethylene oxide, formaldehyde, hydrogen peroxide, and chlorine dioxide are four major commercially available vapor phase sterilization techniques used by the medical and food processing industries. A major disadvantage with ethylene oxide and formaldehyde techniques is that they leave a residual organic sterilant film on the surface of product material. Because spacecraft must meet strict chemical contamination control requirements, it was decided to evaluate only the hydrogen peroxide and chlorine dioxide techniques.

Hydrogen Peroxide (H₂O₂)

At least two different H₂O₂ sterilization processes are commercially available. One developed by Advanced Sterilization Products (ASP) and the other developed by the Steris Corporation. A typical vapor phase hydrogen peroxide sterilization process developed by ASP. The system injects and vaporizes a solution of 59% hydrogen peroxide into the sterilization chamber at a temperature of 45°C and a pressure of 5 to 10 torr, killing any bacteria on any package and product surfaces the vapor can reach. ASP utilizes a plasma to improve effectiveness of sterilization. At the end of the cycle, free radicals lose their high energy, and the hydrogen peroxide converts to water and oxygen molecules⁽³⁾.

At Steris Corporation, a different approach is used to inject hydrogen peroxide⁽⁸⁾. Air saturated with H₂O₂ and reduced humidity is injected into a flash evaporator then into the sterilizer chamber. Generally, fans are used to circulate air and assure uniform distribution of hydrogen peroxide. A vacuum process has also been described^(8, 9).

Both approaches work effectively. It has been demonstrated that a vacuum cycle provides better penetration into convoluted and hard to reach surfaces than aeration alone. This approach will have specific application to sterilization of large exposed areas of a spacecraft or spacecraft assembly areas.

Chlorine Dioxide (ClO₂)

In one commercial system, chlorine dioxide is generated in situ by the action of chlorine on sodium chlorite⁽¹⁰⁾. The chlorine is presented as 2% Cl₂ in N₂. The generator

employs a two-column system, with discharge of the chlorine into the first column pressure controlled and monitored, and output from the generator monitored by a fiber-optic UV absorption system. The working life of the column is limited to 70% of its theoretical capacity, as established by validation studies, to ensure that the conversion process will always take place effectively. The second column is used as a backup.

The ClO₂ sterilizer is operated at slightly above room temperature (32°C), which allows for good control over the process. The process cycle begins with a vacuum air-removal stage followed by a dynamic conditioning stage to humidify the chamber and load to an RH of about 70% or more. At the end of the conditioning phase, ClO₂ gas is introduced to give a concentration of 30 mg/L. This is then topped off by the addition of N₂ at pressures of 80 kPa. A total gas exposure time of about 60 minutes is standard. At the end of the cycle, the ClO₂ is removed using a four-pulse dynamic air exchange.

Advantages of this process compared with ethylene oxide and formaldehyde is that there is no residual sterilant left on the surface of product material, and ClO₂ is not flammable in air.

Gaseous ClO₂ may be removed from the effluent stream by scrubbing with Na₂S₂O₃. Residual levels for discharge to the atmosphere can be well below 1 ppm and are usually undetectable.

The gaseous chlorine dioxide system is currently being used in several medical applications and was recently employed to decontaminate a government building in Washington, D.C. ClO₂ is considered as a highly corrosive chemical, and its compatibility with various spacecraft materials has yet to be demonstrated.

CERTIFICATION PROCESS

Selection of a Sterilization Technique

Selection of a sterilization technique by NASA is based on ease of application, availability of lethality data, and compatibility with spacecraft materials.

The two techniques described above, vapor phase hydrogen peroxide and vapor phase chlorine dioxide, were assessed on these criteria through a comprehensive review of the available literature⁽¹¹⁾.

Selection Based Upon Ease of Application: Studies performed by Krebs, et al⁽¹²⁾ demonstrated that H₂O₂ plasma is not bactericidal, but is effective for rapid removal of residual H₂O₂. It is believed that plasma, based on chemistry, only helps in decomposition of residual H₂O₂ to harmless and stable water and oxygen molecules. Free radicals (OH and HO₂) formed are short lived and they only accelerate further decomposition of hydrogen peroxide. For these reasons, current

discussion will concentrate only on vapor phase H₂O₂ and ClO₂, excluding plasma.

Application of both biocides includes the following phases: conditioning, charge and diffusion, exposure (sterilization), and aeration. Vapor phase hydrogen peroxide (VPHP) is generated from flash evaporation of a 35 - 50 % solution of H₂O₂, which is available in a variety of safe and easy to handle sizes ⁽¹³⁻²⁸⁾.

Vapor phase chlorine dioxide can be generated via several different chemistries, but a common procedure is the reaction of chlorine gas with sodium chlorite to yield uncontaminated ClO₂ ⁽¹⁰⁾. Generation of ClO₂ requires use of raw materials, some of which are more of a safety hazard than liquid H₂O₂ ⁽¹⁰⁾. Vapor phase ClO₂ has been used successfully to sterilize commercial, medical products ^(29,30).

The final by-products of VPHP are water and oxygen. The by-products of chlorine dioxide can include chlorite, chlorate, chlorine, various acids, and water ⁽¹⁰⁾. Not only is the raw material for generation of H₂O₂ safer to handle than the raw materials for generation of ClO₂, but the by-products of H₂O₂ are safer.

Based upon ease of application, vapor phase hydrogen peroxide is the method of choice for certification as a method of spacecraft sterilization.

Selection Based Upon Availability of Lethality Data:

A review of literature identified more than 60 peer-reviewed articles, patents, or book chapters providing lethality data for VPHP ⁽¹¹⁾. The data included antimicrobial studies on bacteria (vegetative cells and spores), fungi (molds and yeasts), viruses, and parasites. The data confirms that bacterial spores are the more difficult microbial population to kill.

Of the organisms tested to date, *Bacillus stearothermophilus* spores are the most resistant. Utilizing vapor phase conditions of 2mg/L H₂O₂, one cycle at 22°C for 30 minutes in a chamber of 70 ft³ at 20% relative humidity with spores deposited on stainless steel strips, D-values range from 0.5 to 2.0 minutes. Other organisms such as spores of *Bacillus subtilis* var. *globigii*, *B. pumilus*, and *B. circulans* exhibited D-values of less than 0.3 minutes ^(11-13, 18, 21, 25, 27).

A review of literature identified only six articles, patents, or book chapters providing lethality data for gaseous chlorine dioxide ⁽¹¹⁾. Utilizing conditions of 30mg/L ClO₂, one cycle at 30°C for 30 minutes in a chamber of 133 ft³ at 80-85% relative humidity with *B. subtilis* spores deposited on stainless steel strips, investigators obtained D-values of 4.4 minutes ^(10, 11, 29).

Based upon availability of lethality data and results obtained, vapor phase hydrogen peroxide is the preferred method of choice for certification as a method of spacecraft sterilization.

Selection Based Upon Compatibility with Spacecraft

Components: The literature review identified only 25 peer-reviewed articles, patents, or book chapters providing materials compatibility data for VPHP ⁽¹¹⁾. However, those references plus studies from VPHP manufacturers yield a wealth of information on materials compatibility. In summary, VPHP appears to be compatible with the majority of materials used in or on interplanetary spacecraft. While the bulk of data relates to medical materials, the work of Rohatgi, et al ⁽²⁴⁾ is a specific evaluation of spacecraft materials.

The literature review identified only a few peer-reviewed articles, patents, or book chapters providing materials compatibility data for chlorine dioxide ⁽¹¹⁾. Those references, plus studies from ClO₂ instrument manufacturers, yield information on materials compatibility. In summary, ClO₂ appears to be less compatible with many of the materials used in or on interplanetary spacecraft.

Based upon availability of compatibility data and results obtained, vapor phase hydrogen peroxide is the preferred method of choice for certification as a method of spacecraft sterilization.

Research Needed to Certify a Sterilization Technique

The process of certifying a new process as a NASA approved method for spacecraft sterilization involves a number of steps. First is the task of obtaining all the pertinent information on the process. This information includes, but is not limited to, the selection of a standard organism; the development of statistically valid lethality data; the detailed specification of sterilization parameters; the verification of the process using naturally occurring organisms; and the demonstration of the effectiveness of the process at the intended scale of application. Next is the peer review of this material by NASA-appointed experts. The resulting recommendations are then studied by the NASA Planetary Protection Officer (PPO) and his staff who decide on the appropriate implementation and then prepare and present the amended information to the NASA Planetary Protection Advisory Committee (PPAC). The PPAC reviews and makes recommendations on the presented material, with the final step in the certification process being the issuance by the PPO of the appropriate specifications for the new sterilization method.

To pursue this certification, the following have been identified as tasks to be accomplished:

Lethality Data: All lethality data will be collected using hydrogen peroxide sterilization technique (Vacuum + H₂O₂ Vaporization + Pressurization /diffusion + Vacuum and termination of cycle). Considering both the role of plasma in H₂O₂ decomposition and the engineering challenge to uniformly deploy plasma in a room (approximately 10,000 cubic feet) needed to house a

spacecraft, the decision was made to not use plasma in this certification activity.

1. **Microbe Selection:** It was decided that *Bacillus stearothermophilus* and *Bacillus subtilis var. niger* will be used to generate lethality data. JPL will also isolate and identify two more naturally occurring hardy microbes from JPL, KSC, and/or Lockheed Martian Astronautics (LMA) facilities and conduct equivalent experiments on the more resistant microbes to collect additional lethality data. The initial recommendation is to verify the procedure using clean-room isolated strains of *Bacillus pumilus* and *B. circulans*. Early work indicates that the spores from these two isolates are quite resistant to H₂O₂ and thus ideal candidates for the significant challenge of the technique.

2. **Minimum and Maximum Temperature:** The majority of these experiments will be conducted at a minimum temperature of 20°C. This will avoid preheating and temperature gradient problems one may encounter in a heated sterilizer. If sterilizer performance is adequate at 20°C, the use of temperatures higher than 20°C will provide a significantly improved performance at a given H₂O₂ concentration and sterilization cycle time. If sterilizer performance for our intended use is not adequate at 20°C, a temperature between 20 to 45°C should be experimentally established prior to conducting lethality experiments. The maximum temperature of 45°C is recommended by one of the commercial manufacturers and considered adequate to kill 99.99% of the microbial population in approximately 15 minutes⁽²⁰⁾.

3. **Minimum and Maximum H₂O₂ Concentration:** The maximum H₂O₂ concentration that can be achieved in the vapor phase without condensation at 20°C and ~5 torr should be used in these experiments. If it is decided to use 45°C sterilization temperature, a higher H₂O₂ concentration can be maintained resulting in an enhanced microbial reduction. The minimum H₂O₂ concentration value used for these tests is the 1/10 of the maximum value either at 20°C or 45°C.

4. **Microbe Population:** In order to experimentally determine accurate D values, the initial spore population will be large enough to ensure that the remaining microbe population after sterilization is at least 100. It is recommended to use published lethality rate constants to approximately estimate these values.

5. **Surface Types:** Because rate of sterilization is highly dependent upon type of surface on which microbes are deposited, four different material surfaces will be selected for collection of lethality data. Aluminum 6061 will be used to collect all lethality data for all four microbes selected for this task. For the other three surface types (Kapton polymer, polyurethane paint, and epoxied graphite, etc.), lethality data will be collected with only one microbe.

6. **Humidity Effect:** Studies to determine the effect of humidity on rate of sterilization will be conducted. Experiments will also be conducted in which lethality rates are measured for a selected microbe at fixed H₂O₂ concentration, and sterilizer temperature, but in which humidity is varied from 20 to 50% RH.

7. **Calculation of the Lethality Rate Constant:** Because microbial death is a first order reaction, the lethality rate can be expressed by the following equation:

$$dN/dt = -kN$$

or,

$$- \ln (N_t/N_0) = kt$$

Where, N_t is the microbial population at time t and N₀ is initial microbial population. The lethality rate constant is k, which is the slope of line - Ln (N_t/N₀) vs. t.

For a desired log reduction, D values are calculated from lethality rate constants at a specified H₂O₂ sterilizer process condition (hydrogen peroxide concentration, humidity, and sterilizer temperature).

8. **Replicates for Statistical Analysis:** Each experimental point must be replicated five times to establish statistical accuracy of the experimental values.

9. **Microbial Assay:** NASA standard method will be used to estimate the microbial population (N₀ and N_t).

10. **Qualification of Commercially Available Biological Indicators:** After lethality rate data are established and sterilization process conditions are specified, experiments will be conducted to qualify commercially available biological indicators. In these experiments, various biological indicators with verified microbial population (10³ to 10⁸) will be subjected to the sterilization process and performance will be compared to the established lethality rate data. Within lot and between lot evaluations will include sufficient replicates to be statistically significant. A qualified biological indicator will be used to check efficacy of the sterilization cycle and also to evaluate the extent of sterilization of hard to reach exposed surfaces of spacecraft parts.

Sterilizer Scale-up: Commercially-available VPHP equipment is designed for the medical industry and not for spacecraft sterilization. Hydrogen peroxide generators that are used to directly inject the sterilant into a room through its HVAC system (atmospheric pressure application) are available. Such devices will have Planetary Protection applications.

It will be necessary to scale these processes for larger closed chambers (10,000 cubic feet) for terminal sterilization of a spacecraft. A proposed procedure will involve evacuating the chambers to 0.1 Torr; introducing the appropriate concentration of hydrogen peroxide and flash evaporating it to approximately 5 torr, allowing it to diffuse; raising the pressure in the chamber by introducing air, dry nitrogen or argon to 1 atm to "push"

the hydrogen peroxide into the crevices and tubes; waiting to give the sterilant appropriate time to act; and then evacuating the chamber to remove the residual sterilant. The cycle can be repeated until the desired microbe kill rate is achieved.

In this activity a computer model will be developed that will estimate the concentration of the sterilant as a function of the dimensions (x,y,z) inside the chamber for any given geometric configuration and with introduction of sterilant at given locations. Variables will include temperature, relative humidity, chamber pressures, and sterilant concentration. Afterwards, the model will have to be validated by running actual tests.

Assessment of Hydrogen Peroxide Monitors: In order to effectively implement a sterilization process, one has to have an accurate and reliable technique to monitor the concentration of hydrogen peroxide in a sterilizer chamber. There are two commercially available techniques. One is based on UV absorption and the other is based on near infrared absorption. These two methods will be evaluated in the above studies to select the monitor of choice for spacecraft sterilization.

Sterilization of Normal Flora in Spacecraft Assembly Environment: After all lethality data are collected and sterilization process conditions are well established, a study will be conducted to demonstrate that specified process conditions are effective to sterilize normal microbe fallout collected from various spacecraft assembly facilities (JPL, KSC, and/or LMA).

CONCLUSION

A vapor phase hydrogen peroxide sterilization process is recommended as a low temperature alternative to dry heat for terminal sterilization of spacecraft. This selection is based on ease of application, availability of lethality data, and compatibility with spacecraft components. This paper has outlined research that will be conducted to achieve certification of this technique for terminal spacecraft sterilization.

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