# **Decontamination Methods Trade Study**

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#### Introduction

The following trade study was done to answer the following task from the Sandia JPL Collaboration for Europa Lander Statement of Work:

Perform a trade study to assess the feasibility of other sterilization/decontamination methods for reducing forward biological contamination on S/C and assess their suitability for PP applications.

### Scope

For the alternative sterilization methods we focused on current technologies for gas phase sterilants and non-chemical sterilization. Gaseous chemical sterilants were characterized in terms of dose, contact time required, physical properties, environmental parameters known to be required for effective sterilization and finally, the likely effect on spacecraft electronics. The results of this literature survey are summarized in Table 1. Sterilization parameters were based on the sterilization of gram positive spore forming bacteria such as *Bacillus anthracis*, *B. pumilus*, *B. atrophaeus*, *B. globigii* and *Geobacillus stearothermophilus*. Although each of these species may vary in the rate of inactivation by any given sterilant, these data should give a general indication of the properties of each sterilant and its potential suitability for in flight sterilization of the spacecraft vault.

#### **Decontamination Methods Studied**

Several sterilants can be rejected out of hand for having known environmental requirements that are unlikely to be found in the spacecraft vault during flight. The most significant of these is a requirement for high relative humidity. Sterilants that were rejected on that basis include ethylene oxide vapor (Schley et al 1959, Ernst and Dole 1968), peracetic acid vapor (Malchesky 1993), beta propiolactone vapor (Hoffman et al 1958) and ozone (Menetrez et al 2009, Ishizaki et al 1986). Very little literature was found for the use of chorine for gas phase sterilization, with one recent study on the combination of high temperature, high humidity and chlorine gas increasing the efficiency of thermal inactivation for bacterial spores (Zhou et al 2016). Chlorine gas is known to be incompatible with electronics.







Other sterilants have drawbacks that may make them less than optimal for this application. Hydrogen peroxide vapor (Pottage et al 2012, Rastogi et al 2009), a commonly used sterilant for high containment facilities (biosafety level 3 or 4) may be insufficiently stable for use after long periods of storage during spaceflight and may not perform well under conditions that would freeze water. Methyl bromide is known to have a low dose requirement, but the contact time (24 hrs) is on the high end of those reported (Kolb et al 1950). Propylene oxide proved to be less than 100% effective at killing spores (Bruch et al 1960).

From this analysis, one can conclude that perhaps the three most promising chemical sterilants for rapid in-flight sterilization of the vault are chlorine dioxide (Pottage et al 2012, Rastogi et al 2009), nitrogen dioxide (Noxilizer Inc.) and paraformaldehyde gas (Taylor et al 1969). All 3 have been deemed safe with most materials according to information provided by the manufacturer and may be compatible with non-destructive sterilization of spacecraft components during flight. There is a substantially longer history of chlorine dioxide use as a sterilant. Published values indicate that 40 grams of chlorine dioxide would be sufficient to sterilize a 1 cubic meter vault. Nitrogen dioxide is the most recent addition to the suite of gas phase chemical sterilants. The attractiveness of nitrogen dioxide is the combination of low concentration and low contact time required. Manufacturer values suggest that 3 grams of nitrogen dioxide would be sufficient to sterilize a 1 cubic meter spacecraft vault.

Paraformaldehyde gas is another common sterilant used for the decontamination of biosafety cabinets prior to the replacement of HEPA filters (Taylor et al 1969). Paraformaldehyde gas is usually produced in situ from the relatively rapid depolymerization of the solid material by heat. Under standard pressure conditions, this is carried out a 232°C. An advantage of this method of sterilization is the stability, ease of handling, and ease of storage of the solid material and the relatively low dose and contact time required for sterilization. The major disadvantage is the energy input required to depolymerize the solid. Approximately 10g would be necessary to decontaminate a 1 cubic meter vault. This amount of material can be depolymerized in less than 1 minute.

The major caveat in this analysis is that no data have actually been reported for the activity and effectiveness of any chemical sterilant under conditions that approach those of the vault during spaceflight. The most significant of these is the low temperature (-40°C) that is expected in the vault during spaceflight. Most reports consider 20-25°C to be "low temperature". The warming of the vault prior to sterilization is indeed a possibility, but the energy and weight required to do this is beyond the scope of this analysis.

Three non-chemical sterilization methods were evaluated: ultraviolet irradiation (Vesley et al 2000), gamma irradiation (Horne et al 1959) and heat. U.V. irradiation is a common method for killing microorganisms, however, it is markedly less effective on spores and all surfaces to be decontaminated must be in line of site of the UV source. The energy required for sterilization of the vault would be dependent on the number and strength of individual U.V. sources required to illuminate all surfaces. Gamma irradiation is a well characterized method of sterilization with 1X10<sup>6</sup> rad required to kill bacterial





spores and has the advantage of penetrating the less dense spacecraft components, limiting the number of sources that must be included in the space craft.

Heat, in the form of incineration is the most likely form of destructive non-chemical sterilization to be applied. This method has a long history of effective use and is a principal method of destroying infectious materials (Peterson and Stutzenberger 1969, Barbeito, M.S. and Gremillion, G.G. 1968). Standard incinerators operate at temperatures of 600-1000°C. According to 1 report, incinerator residence times as low as 12 seconds are sufficient to destroy bacterial spores (Allen et al 1989). More recent work on rapid thermal pulses has shortened the time required for the inactivation of spores to sub seconds. This is dependent on a rapid temperature ramp rate of 10<sup>4</sup>-10<sup>5</sup>°C/sec. The actual parameters for sterilization will be dependent on the rate of heat production and the thermal properties of the surfaces to be decontaminated.

Finally low temperature (below 70 °C) atmospheric plasma mixtures for sterilization is a developing area. Plasmas are created by the electrical discharge of a gas. Several gas mixtures (e.g. helium with 2% oxygen) and ionization methods (e.g. radio frequency of 13-56MHz) have been used (Hong et al 2008). Most of the literature is in regards to decontamination of surfaces using a plasma jet, and it is unclear as to whether this is applicable to a 1 cubic meter space. There are not currently any commercial processes available (Shintani et al 2010).

Laboratories



| Sterilzation method       | Dosage            | Time reguired                                                                            | temperature requirements/effects                                | Other Limitations                                                 | Electronics compatiblilty | References                                                                                                        |
|---------------------------|-------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------|---------------------------|-------------------------------------------------------------------------------------------------------------------|
| Ethylene Oxide Vapor      | 400-800 mg/l      | 35-60 minutes                                                                            | 10 degree drop in temperature requires doubles the contact time | relative humidity 30-60%                                          | Safe with most materials  | Schley et al 1959, Ernst and Dole 1968                                                                            |
| Chlorine Dioxide          | 40 mg/l           | 60 Minutes at 25 °C                                                                      | Melting point - 59 °C<br>Boiling point 11 °C                    |                                                                   | Safe with most materials  | Pottage et al 2012, Rastogi et al 2009                                                                            |
| Chlorine Gas              | 0.3 mg/L          |                                                                                          | fast heating to 300 C° 40-100% humidity                         |                                                                   | Incompatible              | Zhou et al 2016                                                                                                   |
| Hydogen Peroxide Vapor    | 2.4 mg/           | 8 to 60 minutes at upt to 50 C                                                           | Melting point 0.43 °C<br>Boiling point 150. 2 °C degree         | spores required contact time of greater than 20 minutes           | Safe with electronics     | Pottage et al 2012, Rastogi et al 2009                                                                            |
| Nitogen Dioxide Gas       | 3.0 mg/L          | 5 minutes                                                                                | Melting point -11.2 °C<br>Boiling point 21 °C                   |                                                                   | Safe with most materials  | Noxilizer Inc. 2012                                                                                               |
| Paraformaldehyde Gas      | 10 mg/l           | 60-180 minutes at 23 C                                                                   | 20 g/min depolymerized at 232 °C                                | relative humidity above 60%                                       | Safe with most materials  | Taylor et al 1969                                                                                                 |
| Ozone                     | 3.0 mg/L          | innefectve against spores                                                                |                                                                 | requires highhumidity                                             | Safe with most materials  | Menetrez et al 2009, Ishizaki et al 1986                                                                          |
| Peracetic Acid            | 1 mg/l            | 10 min                                                                                   | Melting point boiling 0 °C<br>Boiling point 77 °C               | Regires high humidity                                             | Safe with electronics     | Malchesky 1993                                                                                                    |
| Beta Propriolactone Vapor | 1.5 mg/L          | 2 minutes at 27 C<br>>300 minutes at -10C                                                | Melting point -93.66 °C<br>Boiling point 38.41 °C               | Ineffective at low relative humidity                              |                           | Hoffman et al 1958                                                                                                |
| Methyl Bromide            | 3.4-3.9 g/l       | 24 h                                                                                     | Melting point 0.43 °C<br>Boiling point 150. 2 °C                |                                                                   |                           | Kolb et al 1950                                                                                                   |
| Propylene Oxide           | 1000 mg/l         | 3 h                                                                                      |                                                                 | 3.7 % survival                                                    |                           | Bruch et al 1960                                                                                                  |
| Plasma He/O2              |                   |                                                                                          |                                                                 |                                                                   |                           | Hong et al 2008, Shintani et al 2010                                                                              |
| U.V. Irradiation          | 40 microWatts/cm2 | 10-30 min                                                                                |                                                                 | Less effective against spores. Must be in line of sight of source |                           | Vesley et al 2000                                                                                                 |
| Gamma Irradiation         | 1 X106 Rad        |                                                                                          |                                                                 |                                                                   |                           | Horne et al 1959                                                                                                  |
| Heat                      | 600-1000 °C       | sub second pulse at 700 °C or<br>12.5 second residence time at<br>1000 °C in incinerator |                                                                 |                                                                   |                           | Peterson and Stutzenberger 1969,<br>Barbeito, M.S. and Gremillion, G.G. 1968<br>Allen et al 1989, Zhou et al 2015 |





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