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**Subject: Unpublished Data Results with Bioxy- as Virucidal Shared with Atomes, Inc.**

Background: For past five years, Dr. Rastogi lab has been investigating sporicidal, bactericidal and virucidal potential of Bioxy solution. The concentration range tested have been 0.5% through 5% (wt./vol.). Typically 100 ml solution is prepared each time, and the powder is dissolved at room temperature for an hour before use. The solution is tested within 2-3 hours after preparation. Neutralizer used for inactivating the active components included 2% sodium thiosulphate.

Bioxy efficacy has been tested against two viruses, an enveloped (Vaccinia virus, VACV) and a non-enveloped viral (Feline calici virus, FCV) surrogate. The efficacy has also been tested against vegetative cells of *Burkholderia thailandensis* (a surrogate for BW agent *B. mallei*). Finally, the efficacy has been tested against spores of *Bacillus anthracis* (Sterne), a surrogate for pathogenic strain Ames, capable of causing anthrax disease in animals and humans.

The method selected for all disinfection testing was OECD (<http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)11&doclanguage=en>). Briefly, an aliquot of ten microliter is dried on sterilized small circular coupons for 45 - 60 minutes. The dried inoculum is covered with fifty microliter of disinfectant and was allowed to be in contact for ten minutes under ambient conditions. An aliquot of ten milliliter was added after the contact time, and vials containing the coupon were vortexed for one minute to dislodge the inoculum off the surface. Appropriate dilutions were prepared (-1 through -4). For bacterial and spore samples, an aliquot of one hundred microliter was directly spread on tryptic soy plates (in duplicate). For viral samples, infective viral particles was determined by infection in appropriate host cell line, i.e. CRFK (FCV), and Vero (VACV).

**Results:**

**I. Viral Disinfection Runs -**

*I-1. FCV Control Recoveries:*



*I-2. Efficacy of 0.5% Bioxy (10-min contact time) against FCV:*



*I-3. Efficacy of 0.5% Bioxy (10-min contact time) against VACV:*



Bioxy solution (0.5%) was effective against FCV (non-enveloped), was unable to achieve a complete kill on CARC-painted steel and anti-skid surfaces. Control viral recoveries ranged between 5.1 and 5.8. Efficacy of Bioxy was also tested against VACV (enveloped virus) and results are summarized in Figure I-3. The solution was fairly effective on anti-skid, TIS, and steel surfaces.

II. Spore Disinfection Runs -

*II-1. Spore Control Recoveries:*



*II-2. Spore Efficacy: 5% solution with contact time of 30 minutes.*



*II-3. Time-course of 5% solution against Sterne Spores:*



5% solution was tested against spores of *Bacillus anthracis* (Sterne, BaS) strain, with a contact time of 30 min under ambient environmental conditions. The control spore recoveries ranged between 5.5 – 6.3-logs. Three powder versions (Bioxy-H, Bioxy-Enviro, and Bioxy+) were tested, the efficacy was good, but residual spores were recovered from all four surfaces.

III. Vegetative Cells Disinfection Runs -

*III-1. Cells Control Recoveries:*



*III-2. Efficacy of Bioxy against Vegetative Cells:*



Bactericidal efficacy was investigated using vegetative cells of Burkholderia thailandensis. This is a potential surrogate for B. mallei (causing glanders in animals and humans). A 2% solution with a contact time of 15 minute was evaluated as a bactericidal approach. The cell recoveries from control coupons show a range of 5.1 - 6.3-log cells, after drying an inoculum for 60 minutes. The efficacy data show good efficacy on three surfaces, steel, butyl rubber, and anti-skid.

In conclusion, Bioxy appears to demonstrate a strong potential for broad-range bio-disinfectant, effective against viruses, cells, and spores. Unique advantages of Bioxy include, dry decontaminant, non-corrosivity, biodegradable, non-toxic by products, stable shelf life of powder version.

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