

# SYNERGISM AMONG MULTIPLE BIOCIDAL FACTORS ON MARS AND INTERPLANETARY SPACE INCREASES THE LETHALITY OF DIVERSE SPACE ENVIRONMENTS ON SPACECRAFT BIOBURDENS.

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**Introduction:** Recent papers on microbial survival and growth under simulated Martian conditions---and on the potential of microbial proliferation in Special Regions on Mars---have listed between 17 and 22 biocidal or inhibitory factors that will negatively impact spacecraft bioburdens [1,2,3]. The top seven biocidal factors appear to be (in approx. priority order): (i) solar UVC (200-280 nm) irradiation at the surface, (ii) desiccation, (iii) low pressure (6-10 mbar), (iv) anoxic CO<sub>2</sub> atmosphere, (v) volatile oxidants (e.g., O<sub>2</sub><sup>•</sup>, O<sup>•</sup>, H<sub>2</sub>O<sub>2</sub>, etc.), (vi) extremely low temperatures (global average of -61°C), and (vii) potentially biotoxic soils due to high salt concentrations and extremes in acidity/alkalinity in the regolith [3]. The dominant biocidal conditions for interplanetary space are: (a) solar heating on external surfaces, (b) solar UV irradiation on external surfaces, (c) high vacuum, (d) extreme desiccating conditions, and (e) space ionizing radiation [3,4,19].

Multi-factorial experiments are often required to characterize the overarching effects of space and planetary surface environments on microbial survival. However, the number of potential treatments in multi-factorial experiments rises quickly as the number of factors are added. For example, if only three factors are to be considered, there is an experimental landscape composed of 6 possible treatments (3! = 6). If four factors are to be considered, the experimental landscape balloons to 24 possible combinations (4! = 24). Etc. Thus, multi-factorial experiments seldom exceed two or three factors.

The goal here is to briefly review the literature for synergistic interactions of biocidal factors that are relevant to the surface of Mars or interplanetary space and propose three key Strategic Knowledge Gaps (SKGs) in which new simulations are encouraged.

**Synergism between UV irradiation and vacuum.** In all cases reviewed here, survival rates were significantly lower by 2 to many orders of magnitude for vegetative cells and endospores of multiple species exposed simultaneously to simulated solar UV irradiation and high vacuum compared to either factor alone. Examples: (i) Bucker et al. [5] found that *Bacillus subtilis* spores exposed to both factors were inactivated at a rate of 2 logs faster per time-step compared to single factors alone. (ii) Similar results were reported for *B. subtilis* by other authors [6,7,8]. And (iii), Keller and Horneck [9] reported diverse strains of *Streptomyces griseus* exhibited between 1/2 and 3 orders of

magnitude increased lethality when high vacuum plus low levels of solar UVC irradiation were combined. What is unusual about the Keller and Horneck study was the high levels of biocidal activity when the UV fluence rate was only 100 - 200 J/m<sup>2</sup>. A UV flux of only 200 J/m<sup>2</sup> is accumulated in only a few minutes on the surface of Mars [4,10,11]. And lastly, Diaz and Schulze-Makuch [12] demonstrated survival rates for *Deinococcus radiodurans* decreased from 67% for UV alone to < 0.1% for a combined exposure of UV and vacuum.

**Synergism between heat and vacuum.** When considering the combined effects of solar heating plus vacuum, the synergism can equal or exceed those rates observed for UV plus vacuum. Examples: (i) Schubert and Beaudet [13] demonstrated between 1 and 5 log reductions of synergism when *Bacillus* spp. spores were exposed to the combined effects of heat (up to 170°C) and vacuum (< 1.0 mbar). (ii) Schuerger et al. [4] demonstrated a > 6 log reduction when spores of *B. subtilis* were exposed to 10<sup>-6</sup> mbar and 100°C. And (iii), Hagen et al. [14] reported increased lethality was positively correlated to higher temperatures for multiple species including *B. subtilis* var. *niger*, *Micrococcus* sp., and *Staphylococcus epidermidis*.

**Synergism between ionizing radiation and vacuum.** Only one paper [15] was found that tested microbial survival during concomitant exposures to ionizing radiation and vacuum. Surprisingly, the synergism was opposite what might be expected; namely, survival of *B. megaterium*, *B. subtilis* var. *niger*, *Clostridium sporogenes*, *Aspergillus niger* spores were all better by 1-2 orders of magnitude when spores were exposed to 10<sup>-9</sup> mbar and up to 5 kGy of gamma-radiation compared to the same dosage in air at 1013 mbar. The increased survival in vacuum was likely due to the loss of ionized free radicals (e.g., O<sub>2</sub><sup>•</sup>, O<sup>•</sup>, NOx<sup>•</sup>) in the high-vacuum conditions compared to room air and pressure.

**Synergism between UV irradiation and temperature.** Few papers were found in the literature that explored synergism between other factors possibly encountered on Mars. One exception is the work by Ashwood-Smith and Horne [16] that showed significant protection against UV irradiation for spores of the fungus, *Aspergillus nidulans* when temperatures were extremely low (-196°C versus 23°C).

**Synergism between Mars surface conditions, UV irradiation, and biotoxic soils.** And finally, Schuerger et al. [17, 18] demonstrated that combinations of the three

factors listed above can significantly increase the lethality of Martian regolith between 0.5 logs (*B. subtilis*) and 7 logs (*Enterococcus faecalis*). The lethality was significantly increased when soil solutions [18] were used compared to desiccated soils [17].

**Synergism among diverse factors for planetary surfaces.** Recently, a Lunar Microbial Survival (LMS) model [4] was proposed that predicted extremely fast lethality rates for external spacecraft surfaces on the Lunar surface. Combining the effects of solar heating, solar UV, vacuum, and ionizing radiation in cis-lunar space, the LMS model predicted that as many as 2479 log reductions might be plausible for external spacecraft surfaces over one Lunar day (i.e., 14.77 days) at the equator.

What does a prediction of 2479 log reductions per lunation actually mean? First, the average bioburdens on Lunar spacecraft are approx.  $4.57 \times 10^{10}$  spores or cells per vehicle [4]. If we use the industry definition of one Sterility Assurance Level (i.e., SAL) equals a 12-log reduction [16], then 2479 divided by 12 = 207 SAL cycles are achieved for external spacecraft surfaces on the Moon each and every Lunar day. Rates of inactivation on internal surfaces are slower because solar UV and solar heating are attenuated. However, the external surfaces are quickly sterilized many times over during each lunation.

Furthermore, a Cruise-Phase Microbial Survival (CPMS) model for interplanetary spacecraft [19] predicted that synergistic processes among vacuum, solar heating, and solar UV dominate the biocidal effects for external surfaces out to 10 AU of the Sun (i.e., just beyond Saturn). In contrast, vacuum and temperature are the most important processes for internal surfaces within 10 AU of the sun. Beyond 10 AU, vacuum alone dominates the biocidal nature of interplanetary space. Based on the analysis of the CPMS, both the Cassini and Galileo spacecraft were likely free of viable bioburdens at atmospheric reentry used for disposal.

In a second example, the Europa Clipper (EC) spacecraft will be launched in October 2024 and spend 3.5 years between Venus and Mars conducting one Earth and two Venus flybys before entering into a trajectory to Jovian space. If we assume that 207 SALs will be achieved every 14.77 days when a spacecraft is close to 1 AU (i.e., based on the LMS model [4]), then external surfaces of the Europa Clipper spacecraft are likely to accumulate 17,904 SALs before leaving the inner Solar System. By any measure of lethality, the synergistic effects of solar heating, solar UV, interplanetary vacuum, and ionizing radiation will render the EC external surfaces sterile within a very short period of time.

**Strategic Knowledge Gaps (SKGs).** The following three SKGs may help constrain the science of synergism

of interacting biocidal factors in order to lower the risks of forward contamination of Mars and Europa.

**SKG-1.** Once humans return to the Moon during the Artemis program, we suggest that spacecraft waste bags from the Apollo missions (i.e., placed under the Lunar Excursion Modules) be targeted to verify the LMS model. The LMS model predicts that the contents of the white nylon waste bags should have been sterilized within a few months to a few years on the Moon.

**SKG-2.** If volatile oxidants on Mars (e.g.,  $O_2$ ,  $O$ ,  $H_2O_2$ ) working synergistically with other factors can permeate spacecraft hardware, the rates of inactivating internal spacecraft surfaces might be adequate to sterilize internal structures. Diffusion rates of volatile oxidants into equipment under robust multi-factorial Mars simulations might confirm internal spacecraft sterilization rates over moderate time-steps of several to a few hundreds of sols.

**SKG-3.** Similarly, the concentrations and rates of diffusion of volatile oxidants on Mars through regolith (plus the direct biotoxic nature of regolith [17,18]) would also help constrain the sterilization rates of buried equipment or the undersides of landed hardware in contact with the surface.

**Conclusions.** Synergistic interactions of multiple biocidal factors on Mars and in interplanetary space have the capacity to significantly enhance the lethality of these environments on spacecraft bioburdens. Due to the complexity of conducting multi-factorial experiments---especially when treatments exceed 3 parameters---single-factor experiments are often preferred for simplicity. Thus, it is likely that the 'true' biocidal natures of the Martian surface and interplanetary space are currently being under estimated because experiments are limited, in general, to single-factor experiments.

**References:** [1] Rummel et al. (2014), *Astrobiology*, 14, 887-968; [2] Stoker et al. (2010), *JGR*, 115, E00E20, 1-24; [3] Schuerger et al. (2013), *Astrobiology*, 13, 115-131; [4] Schuerger et al. (2019), *Astrobiology*, 19, 730-756; [5] Buckner et al. (1974), *Life Sci. Space Res.*, 7, 209-213; [6] Weber and Greenberg, (1985), *Nature*, 316, 403-407; [7] Horneck (1993), *Origins Life Evol. Biosphere*, 23, 37-52; [8] Buckner et al. (1971), *Biophysik*, 7, 217-222; [9] Keller and Horneck (1992), *J. Photochem. Photobiol. B: Biol.*, 16, 61-72; [10] Schuerger et al. (2006), *Icaurs*, 181, 52-62; [11] Schuerger et al. (2003), *Icarus*, 165, 253-276; [12] Diaz and Schulze-Makuch (2006), *Astrobiology*, 6, 332-347; [13] Schubert and Beaudet, (2011), *Astrobiology*, 11, 213-223; [14] Hagen et al. (1971), *Space Life Sci.*, 3, 108-117; [15] Silverman et al. (1967), *Appl. Microbiol.* 15, 510-515; [16] Craven et al. (2021), *Intern. J. Astrobiol.*, 20, 1-18; [17] Moores and Schuerger (2020), *Astrobiology*, 20, 1450-1464.