Biocidal Properties of Europa Lander Solid Rocket Motor Adhesive, Loctite® EA9394, Against *Hypsibius dujardini*

Natalie R. Williams, Mark R. Liles, and Morgan R. Sisk

An ice-covered moon of Jupiter, Europa is a prime candidate as a potentially viable environment in NASA's search for extraterrestrial life. Since Europa is a celestial body unlike any NASA has explored before, new issues regarding forward contamination of Europa and sterility of the spacecraft arise. These novel problems that often accompany exploration of new frontiers must be solved in accordance with NASA planetary protection standards, and launch of the Europa Lander is ultimately contingent upon the fulfillment of these criteria.[1]

The Europa Lander Mission launch criteria requires spacecraft decontamination of terrestrial microorganisms to limit the probability of contaminating Europa's surface. These standards include reducing microorganisms present on the lander by at least 10^{-6} and a final Europan contamination probability of 10^{-4} %.[2] For the mission to be a success, the microbiome of certain spacecraft problem areas must be quantified, and the inherent antimicrobial or biocidal properties of native rocket motor materials must be characterized. We discovered that a frequently used rocket motor adhesive, Loctite® EA9394, can reduce the number of viable *Bacillus atrophaeus* vegetative cells by 10 million-fold, and spores by greater than 100-fold, after short-term exposure. Though spore-forming bacteria have traditionally been the primary targets of planetary protection efforts, eukaryotic *Hypsibius dujardini* (i.e., tardigrades, or "water bears") is the best animal model for planetary protection given their known resistance to radiation, desiccation, and space environments.[2] This study evaluated susceptibility of *H. dujardini* to exposure to Loctite® EA9394.

Viability assays were performed with populations of active *H. dujardini* in water and several concentrations of water-soluble extracts of Loctite® EA9394 using 70% ethanol as a positive control and spring water as a negative control. Final concentrations of extracts in solution ranged from 1.6 mg/mL to 0.025 mg/mL. After 24 hours, each replicate was observed under a microscope at 100X magnification. Immobile tardigrades were classified nonviable, while spontaneously mobile tardigrades were classified viable.

We found that increasing adhesive concentration correlated with an increase in tardigrade death. There was a statistically significant difference of percent viability from 1.6 to 0.8 mg/mL of extract when compared to negative control, corresponding to 100% and 94.9% viability reduction, respectively. In summary, active *H. dujardini* populations suffered an average of 100% viability loss when exposed to 1.6 mg/mL of water-soluble extracts for 24 hours. The minimum inhibitory concentration at which 50% viability was observed (MIC_{50}) was approximately 0.2 mg/mL.

The results of this study suggest the risk posed by radiation and desiccation-resistant *H. dujardini* could be mitigated through the biocidal activity of Loctite® EA9394. These reductions can be incorporated into the launch risk assessment for the Europa Lander Mission. As this is an ongoing project, next steps include inducing anhydrobiosis in *H. dujardini* and testing these dormant, extremotolerant populations against Loctite® EA9394 to calculate percent viability reductions as done before, as well as identifying the sporicidal and/or tardigradicidal compounds in the adhesive using mass spectrometry.

Statement of Research Advisor

Natalie Williams has made very significant contributions to the NASA Europa Lander mission in identifying an adhesive that can be used to reduce microbial contamination of the solid rocket motor insulation. She has developed many skills in quantifying adhesive effects on viable numbers of bacterial spores and tardigrades, and these data will be very useful for NASA's planetary protection mission.

– *Mark Liles, Biological Sciences*

References

[1] National Aeronautics and Space Administration. *NASA HDBK 6022-Handbook for the Microbial Examination of Space Hardware*. National Aeronautics and Space Administration, Washington, D.C. 2010.

[2] National Research Council. *Preventing the Forward Contamination of Europa*. The National Academy Press. 2000.