

Slow degradation of ATP in simulated martian environments suggests long residence times for the biosignature molecule on spacecraft surfaces on Mars

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Abstract

Prelaunch planetary protection protocols on spacecraft are designed to reduce the numbers and diversity of viable bioloads on surfaces in order to mitigate the forward contamination of planetary surfaces. In addition, there is a growing appreciation that prelaunch spacecraft cleaning protocols will be required to reduce the levels of biogenic signature molecules on spacecraft to levels that will not compromise life-detection experiments on landers. The biogenic molecule, adenosine triphosphate (ATP) was tested for long-term stability under simulated Mars surface conditions of high UV flux, low temperature, low pressure, Mars atmosphere, and clear-sky dust loading conditions. Data on UV-induced ATP degradation rates were then extrapolated to a diversity of global conditions using a radiative transfer model for UV on Mars. The UV-induced degradation of ATP tested at 4.1 W m^{-2} UVC (200–280 nm), -10°C , 7.1 mb, 95% CO_2 gas composition, and an atmospheric opacity of $\tau = 0.1$ yielded a half-life for ATP of 1342 kJ m^{-2} ; or extrapolated to approximately 22 sols on equatorial Mars with an atmospheric opacity of $\tau = 0.5$. Temperature was found to moderately affect ATP degradation rates under martian conditions; tests at -80 or 20°C yielded ATP half-lives of 2594 or 1183 kJ m^{-2} , respectively. The ATP degradation rates reported here are over 10 orders of magnitude slower than the UV-induced biocidal rates reported in the literature on the inactivation of strongly UV-resistant bacterial spores from *Bacillus pumilus* SAFR-032 [Schuerger, A.C., Richards, J.T., Newcombe, D.A., Venkateswaran, K.J., 2006. *Icarus* 181, 52–62]. Extrapolating results to global Mars conditions, residence times for a 99% reduction of ATP on spacecraft surfaces ranged from 158 sols on Sun-exposed surfaces to approximately 32,000 sols for the undersides of landers similar to Viking. However, spacecraft materials greatly affected the survival times of ATP under martian conditions. Stainless steel was found to enhance the UV degradation of ATP by over 2 orders of magnitude compared to ATP-doped iridized aluminum, graphite, and astroquartz coupons. Extrapolating these results to global conditions, ATP on stainless steel might be expected to persist between 2 and 320 sols for upper and lower surfaces of landers. Liquid chromatography-mass spectrometry data supported the conclusion that UV irradiation acted to remove the γ -phosphate group from ATP, and no evidence was observed for the UV-degradation of D-ribose or adenine moieties. Long residence times for ATP on spacecraft materials under martian conditions suggest that prelaunch cleaning protocols may need to be strengthened to mitigate against possible ATP contamination of life-detection experiments on Mars landers.

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1. Introduction

NASA's Mars Exploration Program (MEP) (<http://mars.jpl.nasa.gov>; Garvin et al., 2001) and Astrobiology Roadmap (<http://astrobiology.arc.nasa.gov>; Des Marais et al., 2003; Morrison, 2001) have outlined a series of objectives and goals in

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which the search for extant or past life on Mars remains a top priority for near-term robotic missions. Over the next decade, a series of lander and rover missions are planned that will search for extant surface microbiota or residual organic signatures of life on Mars. In order to prevent false-positives in life-detection experiments, and to mitigate against the possible contamination of the planet's surface, planetary protection protocols have been devised to reduce both the microbial bioloads and terrestrial organics on launched vehicles (Space Studies Board, Task Group on Planetary Protection, 1992; Space Studies Board, Task Group on Issues in Sample Return, 1997; Space Studies Board, National Research Council, 2006). Spacecraft sterilization and cleaning procedures include heat, gaseous, and liquid sterilization protocols that are effective in reducing much of the viable bioloads on vehicles (Crawford, 2005; Pflug, 1971; Venkateswaran et al., 2004). However, dormant vegetative cells and spores of terrestrial microorganisms are resistant to many space conditions encountered during the Earth–Mars cruise phase, and it is likely that viable microorganisms on spacecraft components have reached the surface of Mars during recent missions (Fajardo-Cavazos et al., 2007; Schuerger, 2004; Schuerger et al., 2006). Historically, the emphasis in planetary protection has been to reduce the viable bioloads present on payloads and vehicles prior to launch (DeVincenzi et al., 1998; Rummel and Meyer, 1996; Sagan and Coleman, 1965), but more recently, the mitigation of organic biosignature molecules has gained attention (Space Studies Board, National Research Council, 2006; Venkateswaran et al., 2004). In order to maintain the scientific integrity of future life-detection missions to Mars, the short- and long-term fates of both viable microorganisms and their biosignature molecules must be better understood from launch through to the end of the operational missions.

Several recent papers have reported that the high UV fluence rates on Mars are adequate to rapidly inactivate terrestrial microorganism on Sun-exposed surfaces of landed vehicles (Newcombe et al., 2006; Nicholson et al., 2005; Schuerger et al., 2003, 2006). Residence times on spacecraft materials under Mars-normal UV fluence rates for viable spores of the bacterial genus *Bacillus* appear to range from tens-of-minutes to a few hours on sol one after landing (Schuerger et al., 2006). And even the strongly UV-resistant spores of the spacecraft-associated bacterium, *Bacillus pumilus* SAFR-032 (Link et al., 2004), have been shown to be inactivated in as little time as 3 h under clear-sky (optical depth $[\tau] = 0.1$) Mars UV simulations (Schuerger et al., 2006). However, there are many locations within the structures of landed spacecraft in which solar UV irradiation cannot penetrate, and it is likely that some viable microorganisms persist on, or within, Mars landers.

The presence of organic industrial residues, microbial biofilms, and biosignature molecules on spacecraft components are additional factors that must be addressed in any planetary protection program for Mars surface spacecraft. Several recent papers have demonstrated that at least some biosignature molecules can persist for much longer times under martian conditions than cells or spores can retain viability. For example, isolated cells of the cyanobacterium, *Chroococcidiopsis* sp. 029, were fully inactivated after only 30 min under simulated

equatorial Mars surface conditions, but phycobilisomes and esterase activity survived at least 4 h, and DNA and chlorophyll autofluorescence persisted at least 8 h (Cockell et al., 2005). In a second paper, Tauscher et al. (2006) reported that the production of biosignature molecules generated during spore germination events in *B. subtilis* can continue even after cells have been rendered nonviable by exposure to Mars UV irradiation. And several studies (Stoker and Bullock, 1997; ten Kate et al., 2005, 2006) have demonstrated long-term survival of the amino acid, glycine, under simulated martian conditions in which the UV-exposed glycine remained detectable after 250–450 h of continuous UV irradiation.

The importance of long-term survival of organics on Mars has two significant implications for life-detection experiments. First, models on the meteoritic influx on Mars (Bland and Smith, 2000; Flynn and McKay, 1990) suggest that significant amounts of carbonaceous meteoritic materials should be present in the martian regolith. However, the gas chromatography-mass spectrometer (GCMS) instrument on both Viking landers failed to detect any organics in the sampled regolith at the parts-per-billion level (Biemann et al., 1977). Thus, if the organics emplaced on Mars by meteoritic falls are degraded by abiological processes faster than they can accumulate in the regolith, the organic compounds may not be available for a putative extant martian soil microbiota. Second, if organics are totally lacking from the martian regolith, then terrestrial microbial contaminants that might be inadvertently dispersed onto the terrain during robotic surface missions might not have a nutritional base from which to derive sustenance. In either case, the missing organics might preclude the establishment, dispersal, and evolution of both extant martian and introduced terrestrial microbial ecosystems on Mars. In the first case, the missing organics would hinder the search for life on Mars, and in the second case, the missing organics would reduce the risk of microbial contamination of the martian terrain.

Several mechanisms have been proposed for the destruction of organic materials on the surface of Mars that include UV irradiation, hydrogen peroxide (H_2O_2), superoxides, and soil oxidants (Bullock et al., 1994; Crawford, 2005; Hunten, 1979; Stoker and Bullock, 1997; Yen et al., 2000; Zent and McKay, 1994). However, little is known on how these mechanisms might affect terrestrial biogenic signature molecules on spacecraft materials under simulated martian conditions. The primary objective of the current work was to investigate the effects of simulated Mars surface conditions, including UV irradiation, on the destruction of adenosine triphosphate (ATP). The biogenic signature molecule was chosen for these studies because it is present in all terrestrial life, and, thus, will likely be present on spacecraft surfaces. Previous research (La Duc et al., 2004; Venkateswaran et al., 2003) has demonstrated that the luciferin/luciferase bioluminescence ATP assay can discriminate between extracellular and intracellular ATP from microbial cells. The current study was constrained to testing the effects of martian conditions on the degradation of extracellular ATP as a gauge of how long the biogenic signature molecule might persist on spacecraft components under Mars surface conditions. Other studies have been completed on the persistence of intra-

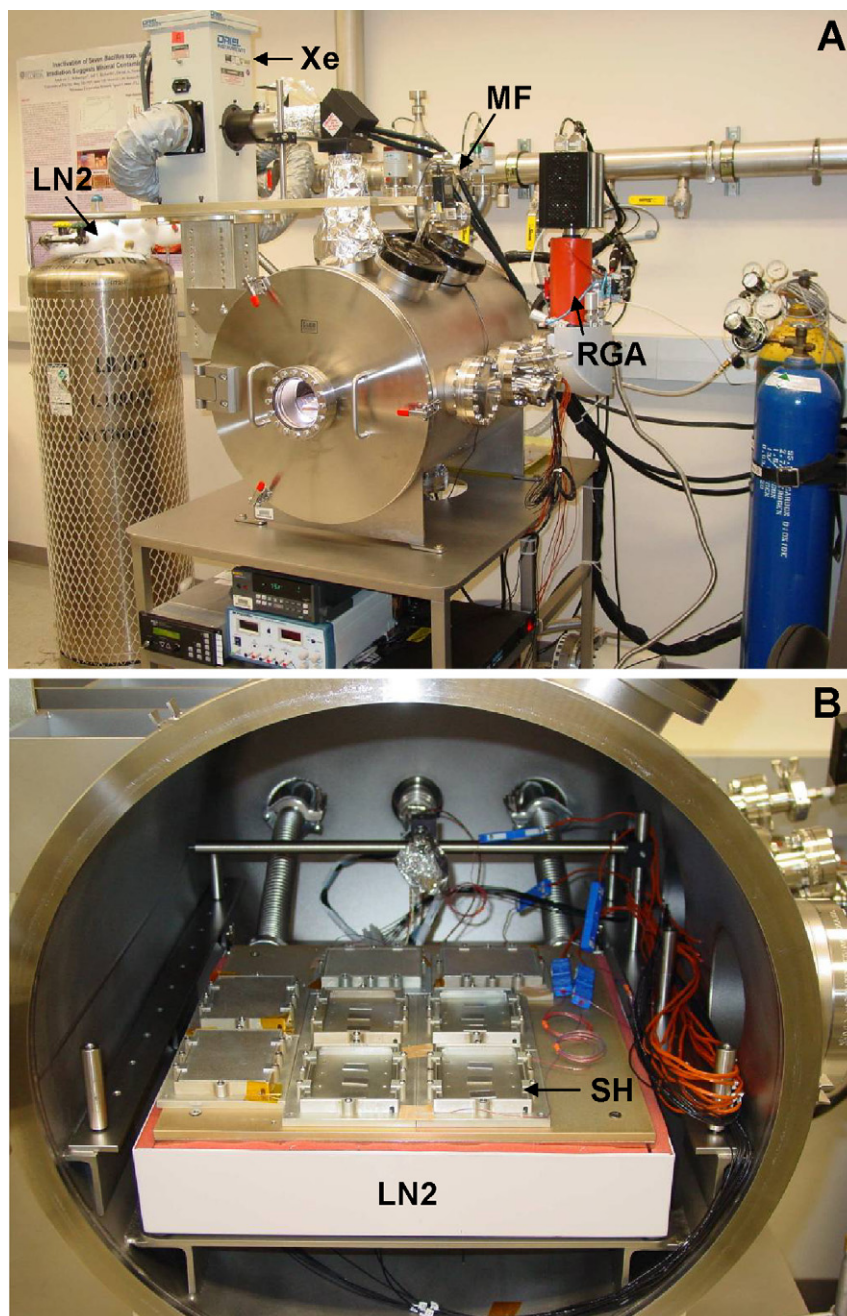


Fig. 1. Mars Simulation Chamber (MSC). (A) The MSC system used a xenon-arc (Xe) lamp to deliver UV irradiation to the samples. The Mars gas mix (blue tank on the right) was injected into the MSC system using a mass-flow (MF) controller. Environmental data loggers were positioned below the MSC system or off-camera to the right. Liquid nitrogen (LN2) tanks were positioned to the left of the MSC and fed the LN2 thermal control plate through ports in the rear of the MSC. (RGA = residual gas analyzer.) (B) The LN2 thermal control system was positioned at the front of the MSC; LN2 lines are seen at the rear of the chamber. The UV irradiation was calibrated to simulate Mars UV fluence rates on the tops of spacecraft coupons positioned within sample holders (SH) mounted on top of the LN2 thermal control plate. Environmental sensor wires can be seen on the right of the internal view of the MSC.

cellular ATP in bacterial cells under martian conditions, and will be published elsewhere.

2. Materials and methods

2.1. Mars Simulation Chamber

A Mars Simulation Chamber (MSC) was developed as part of an ongoing series of Mars astrobiology and planetary protec-

tion projects (Schuerger's lab) (Fig. 1). The MSC is a cylindrical stainless steel low-pressure chamber with internal dimensions measuring 70 cm long by 50 cm in diameter (built by LACO Technologies, Salt Lake City, UT, USA). All vacuum fittings were obtained from MDC Vacuum Products, Corp. (Hayward, CA, USA), and the vacuum pump was from Varian Vacuum Technologies (model SH100 scroll pump, Lexington, MA, USA). Pressure setpoints were maintained with an electronic pressure controller and throttle valve (models 6521D and

253B-1-2CF-1, respectively; MKS Instruments, Inc., Andover, MA, USA). Atmospheric gases were delivered to the MSC from commercially obtained tank mixes (Boggs Gases, Titusville, FL, USA) through a mass-flow controller. A liquid-nitrogen (LN2) thermal control system (model TP1265, Sigma Systems Corp., San Diego, CA, USA) served as the primary temperature control system for the MSC. The LN2 thermal control plate was fully programmable between -100 and $+200$ °C, including ramping characteristics for controlling diurnal temperature changes. Mars-normal UV-vis-NIR irradiation was supplied to the inside of the MSC through fused silica glass ports (model 9722009, Insulator Seal, Inc., Sarasota, FL, USA). Ultraviolet irradiation was produced with one, 1000 W xenon-arc lamp (model 6269, Oriel Instruments, Stratford, CT, USA), and calibrated to deliver Mars UV fluence rates (sensu Schuerger et al., 2003, 2006). A 6-cm water filter was inserted into the light path just after the xenon arc lamp housing to attenuate NIR and mid-IR irradiation. The water filter effectively removes all thermal irradiation above 1100 nm. The spectrum of the UV light (200 to 2500 nm) has been published (Schuerger et al., 2003, 2006). The MSC system can accurately simulate five key components of the surface environment of Mars including: (a) pressures down to 0.1 mb, (b) UVC, UVB, and UVA irradiation from 190 to 400 nm, (c) dust loading in the atmosphere from optical depths of 0.1 (low-dust sky) to 3.5 (global dust storm) using a series of neutral density filters (described by Schuerger et al., 2003), (d) temperatures from -100 to $+30$ °C [based on Viking and Mars Pathfinder data (Golombek et al., 1999; Keiffer, 1976)], and (e) a gas mixture composed of the top five gases in the martian atmosphere [CO_2 (95.54%), N_2 (2.7%), Ar (1.6%), O_2 (0.13%) and H_2O (0.03%); based on Viking data (Owen, 1992)]. All optical depths are given as total atmospheric opacities at 671 nm, and are composed of dust, ozone, and CO_2 components (see Section 2.5).

The mean UVC (200–280 nm) fluence rate within the Mars chamber was calibrated at 4.1 W m^{-2} ($\pm 0.2 \text{ W m}^{-2}$) within the ATP sample holders (Fig. 1B), which delivered $14.8 \text{ kJ m}^{-2} \text{ h}^{-1}$ of UVC irradiation to the ATP-coated coupons placed on the LN2 thermal control plate. The mean UVB (280–320 nm) and UVA (320–400 nm) fluence rates on the spacecraft coupons were approximately 5.6 and 15.8 W m^{-2} , respectively. The UVC fluence rate used herein compares favorably to the fluence rates predicted by a number of Mars UV models (Kuhn and Atreya, 1979; Cockell et al., 2000; Patel et al., 2004; Moores et al., 2007; Schuerger et al., 2003). The UVB fluence rate also compares favorably to these models, but the UVA flux is slightly lower than predicted. However, a key assumption of this research is that UV-degradation of ATP is likely caused by UVC photons in the range of 200–250 nm (see discussions by McLauren and Shugar, 1964), thus, these experiments were aligned with current UV models for Mars.

Based on the Mars UV model described by Moores et al. (2007), a UVC fluence rate equal to a worst-case scenario on Mars would equal approximately 3.4 W m^{-2} instantaneous UVC flux as measured on a flat horizontal surface at 22° S latitude, $L_s = 250^\circ$ (perihelion), and $\tau = 0.2$. The maximum daily value of 108.1 kJ m^{-2} is obtained near 80° S at the south-

ern hemisphere summer solstice ($L_s = 270^\circ$). The presence of ozone in the UV transfer models of Moores et al. (2007) pushes the maximum UV flux to a location with the least ozone and longest sol, namely 80° S latitude at the southern summer solstice.

Under the martian simulations reported herein, a daily flux of 108.1 kJ m^{-2} would be accumulated in approximately 7.3 h within the Mars simulator. However, a global average of UVC flux for the equator throughout a martian year would be lower, and, thus, a value of 65.8 kJ m^{-2} was predicted to represent a more realistic picture of how rapidly ATP might be degraded on Mars. A UVC flux of 65.8 kJ m^{-2} would be accumulated within approximately 4.4 h in the Mars simulator, and, thus a 24-h continuous UVC simulation would be similar to approximately 5.5 sols on equatorial Mars on a terrain similar to the Mars Pathfinder (MPF) landing site (Moores et al., 2007). Mars simulations in the current study used continuous UV irradiation to accelerate ATP degradation. This was based on previous research (Schuerger et al., 2003) that indicated that most of the effects of Mars surface conditions on biological samples were created by UV irradiation and exposure times, and in which pressure, temperature, and gas composition had no or minor effects on biological activities. Diurnal changes in UV flux acting directly upon the degradation of organics or by creating diurnal changes in volatile oxidants were not considered in the current study, but may be important processes on the martian surface.

2.2. Spacecraft materials and sample preparation

Spacecraft are assembled using a number of different materials. For most experiments, aluminum 6061 coupons, treated with a chromate conversion film (Iridite 14-2, MacDermid Inc., Waterbury, CT, USA) were used as an experimental standard spacecraft material. The iridized surface (also called chemfilm or alodine) was used because it is a common method for coating aluminum spacecraft parts to reduce corrosion (Schuerger et al., 2005). Additional materials tested were: clear-anodized aluminum; black-anodized aluminum; 304 stainless steel; a graphite/polycyanate composite material (M55J/BtCy-1; hereafter called “graphite”); and a quartz/polycyanate composite material (AQ II/EX1515; hereafter called “astroquartz”). Both the graphite and the astroquartz coupons were obtained from the Jet Propulsion Lab (Pasadena, CA, USA; courtesy of Roger Kern). All coupons were approximately 10×20 mm in size and did not exceed 1 mm in thickness. Metal coupons were heat-sterilized at 130 °C for 24 h and cooled to room temperature before use. The nonmetal materials, astroquartz and graphite, were heat-sensitive, and were sterilized with 70% ethanol and UV irradiation according to a previously published report (Schuerger et al., 2005).

Iridized aluminum coupons were used for all tests as they arrived from the manufacturer (International Tool & Mold, Merritt Island, FL, USA). The Iridite process treats the aluminum coupons with a sulfuric (1.25%) and hydrofluoric (3.75%) acid solution to clean organic residues from the aluminum prior to coating with the chromate conversion film. Extreme care was taken by both the manufacturer and end-user (Schuerger) when

handling the iridized coupons to avoid any recontamination of the coupons. Iridized coupons were handled only with heat-baked forceps with operators using white-nylon or sterile latex gloves. All other coupons were cleaned prior to use by washing them in 1.0 N HCL for 2 min, followed by three separate washes in 18 M Ω sterile double-deionized water.

A 1 μ M solution of disodium ATP (Sigma–Aldrich Chemicals, USA) was prepared in sterile deionized water and 50 μ l of solution were applied to each coupon, yielding 50 picomoles of ATP per coupon. The ATP solutions were air-dried on coupons for 1–3 h at 37 °C. Materials coated with ATP were placed within aluminum sample holders (Fig. 1B; described by Schuerger et al., 2003) that were fitted with either aluminum shields to block all UV irradiation, or fused silica glass filters to create a Mars surface UV simulation of optical depth 0.1 (dust free atmosphere). The sample holders were then placed on the upper surface of the LN2 thermal control plate within the UV light beam of the Mars chamber.

2.3. ATP assay

The ATP assay was based on the work of Venkateswaran et al. (2003) who proposed the use of the ATP-dependant luciferin/luciferase bioluminescence assay for detection of terrestrial microorganisms on spacecraft surfaces during prelaunch processing of payloads and vehicles. Coupons doped with ATP and exposed to martian conditions were separately placed into 13-mm diameter glass test tubes each containing 2 ml sterile deionized water, vortex mixed for 5 s, incubated at room temperature for 5 min, and then vortex mixed again for 5 s. Fifty microliters of each extract were used for subsequent ATP determinations. Extracted ATP was measured using the BacTiter-Glo Microbial Cell Viability Assay (Promega, Madison, WI, USA). Luminescence was detected in a microplate luminometer (Harta Instruments, Gaithersburg, MD, USA) in “glow” mode with a dwell time of 1 s per well. Data are reported in terms of arbitrary relative light units (RLU) as expressed by the microplate luminometer. A standard curve relating ATP concentration to RLU was generated, and all experimental readings fell within the linear range of the standard curve. Deionized water and materials were sterilized and handled in such a way as to avoid contamination with ATP. In preliminary experiments, it was determined that none of the spacecraft materials inhibited or interfered with the ATP assay.

2.4. Experimental simulations of ATP degradation on Mars

Three experiments were conducted on ATP degradation under martian conditions. A series of tests were conducted to determine the effects of temperature on ATP degradation, and two separate experiments were completed to determine if diverse spacecraft materials might enhance or retard ATP degradation under Mars conditions. From the results of these experiments, and using radiative transfer models for UV on Mars (Moore et al., 2007) long-term residence times of detectable ATP on spacecraft surfaces were estimated.

First, ATP-doped coupons were inserted into sample holders and placed upon the LN2 thermal control plate within the MSC system (Fig. 1B). The Mars chamber was sealed, pumped down to 1 mb, back-filled with Mars gas to 7.1 mbar (± 0.1 mb), and equilibrated for 10 min. The LN2 system within the Mars chamber was then started and the samples allowed to stabilize at -80 , -10 , or $+20$ °C (± 1.5 °C) for an additional 20 min. Once the Mars chamber atmosphere was thoroughly mixed with Mars gas, and the temperature stabilized at the appropriate setpoint, UV irradiation from the xenon-arc lamp was allowed to enter the Mars chamber through a fused silica glass port on top of the MSC system, which immediately illuminated the ATP-doped coupons. Experimental controls were composed of (1) ATP-doped coupons exposed to Earth lab conditions of 1013 mbar total pressure, 25 °C, a standard O₂/N₂ atmosphere, and no UV irradiation; and (2) ATP-doped coupons exposed to the martian conditions held within the Mars chamber but shielded from UV irradiation. Coupons were exposed for 0, 2, 4, 7, or 10 days each at the three experimental temperatures, removed from the Mars chamber, and assayed with the Earth controls for ATP.

A second experiment was run to determine the effects of six spacecraft materials (listed above) on ATP degradation under martian conditions. Spacecraft materials were doped with ATP at the rate of 50 μ l of a 1 μ M ATP solution and dried for 3 h at 37 °C. The ATP-doped spacecraft materials were exposed for 7 days under continuous UV irradiation ($\tau = 0.1$) at -10 °C, 7.1 mbar total pressure, and exposed to a Mars gas atmosphere. Coupons were then assayed for ATP, as described above. One set of ATP-doped coupons were assayed at $T = 0$; i.e., on the day the Mars simulations were started. One set of coupons were then exposed to UV irradiation, and one set was not exposed to UV irradiation, under simulated martian conditions.

And third, an experiment was conducted with aluminum coupons to determine if noniridized coupons or heat sterilization could affect the retention or degradation of ATP. The goal was to acquire additional insights into whether handling procedures of spacecraft materials prior to launch could affect ATP recovery from metal surfaces. Untreated aluminum 6061, with and without heat sterilization, and iridized aluminum 6061, with and without heat sterilization, were doped with ATP at the rate of 50 μ l of a 1 μ M ATP solution, dried for 3 h at 37 °C, and immediately assayed for ATP. The coupons were not exposed to martian conditions or UV irradiation. The heat-treated coupons were exposed to 130 °C for 24 h and cooled to 25 °C prior to use.

2.5. Temporal modeling of UV degradation of ATP

A one-dimensional numerical radiative transfer model (recently described by Moore et al., 2007) was designed to estimate the levels of accumulated UV irradiation on unshielded and shielded surfaces of spacecraft and martian surface features. The UV transfer model itself consisted of two distinct layers containing CO₂ and Mie-scattering particulates based upon Mars Pathfinder (MPF) results (Tomasko et al., 1999; Johnson et al., 2003) and consistent with airborne particulates observed by the Viking landers and MER rovers (Lemmon et

al., 2004). The model also contained appropriate levels of ozone which are permitted to vary seasonally and by latitude throughout a martian year according to the simulations of Lefèvre et al. (2004), and recently validated by the SPICAM instrument aboard the Mars Express spacecraft (Perrier et al., 2006). The accumulated incident UVC energy for a martian year was calculated by estimating the incident UV flux for a variety of optical depths and latitudes at forty separate times during each sol, and fitting several sols over the year to an analytic function. From this, the average daily energy was derived and compared to the amount of energy required to achieve a seven half-life (99%) reduction in ATP under various spacecraft configurations and optical depths of dust. The upper surfaces of spacecraft were modeled for latitudes extending from the martian equator to 80° N and S latitude and from optical depths of dust between 0.2 and 5.

To estimate the accumulated UV flux on the undersurfaces of spacecraft, it was necessary to use values for upwelling UV irradiation from the ground (assuming $\tau = 0.5$). This consisted of extrapolating UV reflectance off of the martian surface down to 200 nm from MPF data, and verifying the UV reflectance with laboratory measurements of Mars analog soils (Moore et al., 2007). As a final refinement, the shadow of a square blocking plate centered on the point of interest was added and those altitudes and azimuths of the surface covered by the shadow were blacked out in the final calculation of incident energy. It is the presence of this shadow, particularly at high geometric shielding ratios (GSR), which leads to the low observed UV irradiation levels, and hence, long times to a seven half-life reduction of ATP. The GSR is an expression of the width of the spacecraft undersurface divided by its height above the local terrain. As such, low GSR values are obtained in the case of small lander decks with high ground clearance, and high GSR values are indicative of broad plates held close to the ground. The higher the GSR value, the less rapid would be the accumulated UV irradiation because the undersurfaces of a spacecraft would be exposed to lower levels of reflected direct and diffuse UV irradiation.

2.6. Chemical analyses for ATP breakdown products

Doped coupons were extracted for ATP, and possible UV-degradation products, by placing individual coupons into 5 ml of sterile deionized water in a sealable test tube, and vigorously agitating by hand for 5 min. Samples were analyzed by injecting 5 μ l of solution via an injection loop directly into a 200 μ l min⁻¹ flow of water which was directed into a Thermo Finnigan LCQ-DUO liquid chromatography-mass spectrometer (LCMS) equipped with an ESI interface. The mixture eluted over 0.25 min, leading to a 1:10 dilution of the sample. The interface spray voltage was set at 4.2 kV with a capillary voltage of 43 V and capillary temperature optimized to 145 °C, as described below. The sheath gas and auxiliary gas flows were set at 50 and 52 (arbitrary units), respectively. The circuit current in the interface was typically 3.9×10^{-7} A.

Disodium adenosine triphosphate (Na₂ATP) samples were obtained and stored at -10 °C. Standards were prepared from

the Na₂ATP sample in HPLC grade water (Burdick and Jackson, Inc., Milan, Italy). Direct infusion of a 10 ppm standard was used to tune the LCMS to the m/z 506 peak in negative polarity. The capillary temperature was optimized to 145 °C, where a maximum ATP²⁻ signal was obtained while retaining an ATP²⁻/ADP¹⁻ ratio above 3.

Calibration standards were prepared in the 5–100 ppm range and analyzed to create a calibration curve based on the extracted ion current for ATP²⁻ (m/z 525). The detection limit, defined by a signal/noise ratio of 3 was found to be approximately 20 ppm. Analytical samples from “Mars UV exposure” experiments and nonexposed control samples were received and stored at -10 °C. Samples were warmed to room temperature and analyzed under conditions identical to those used to analyze the standard solutions, as described above.

2.7. Statistical analyses

Statistical analyses were conducted with version 9.0 of the PC-based Statistical Analysis System (SAS) (SAS Institute, Inc., Cary, NC, USA). For the spacecraft materials experiments, data were log- or 0.5 power-transformed prior to analyses to induce homogeneity of variances of individual treatments; all data are presented in the figures as untransformed data. Transformed data from the spacecraft materials experiments were subjected to analysis of variance (ANOVA) procedures (PROC GLM) followed by protected least-squares mean separation tests ($P \leq 0.05$). Data for the temperature experiments were not transformed, and were normalized to the Earth controls on day-zero. Linear regression models were used for analyzing the effects of UV irradiation on ATP degradation at the three temperatures tested, and were generated with PROC REG ($P \leq 0.05$) in SAS. Samples were exposed and assayed in triplicate, and all experiments were conducted twice ($n = 6$).

3. Results

The UV degradation of ATP under simulated Mars surface conditions occurred more slowly than was originally predicted. Initially, we expected that one half-life of ATP would occur in less than 1 Earth day of continuous UV irradiation (i.e., 5–6 sols of simulated Mars UV). However, the UV degradation of ATP at the standard Mars conditions of 4.1 W m⁻² UVC, -10 °C, 7.1 mb, Mars gas, and a $\tau = 0.1$ yielded a half-life of approximately 22 sols for simulated equatorial Mars (i.e., 4 Earth-days in the Mars simulator) (Fig. 2). Thus under these conditions, over 150 sols would be required for a 99% reduction (seven half-lives) in ATP if the biogenic molecule were on a horizontal and fully Sun-exposed surface on the upper deck of a Mars rover. The controls (i.e., Earth and Mars-exposed coupons not treated with UV irradiation) yielded consistent results in which the estimated ATP was not significantly degraded relative to the $T = 0$ samples ($P > 0.10$).

Data from UV-induced degradation of ATP assays (Fig. 2) were normalized to the $T = 0$ Earth controls and replotted with linear regression models (Fig. 3). Temperature was found to affect the rate of ATP degradation, in which the significantly

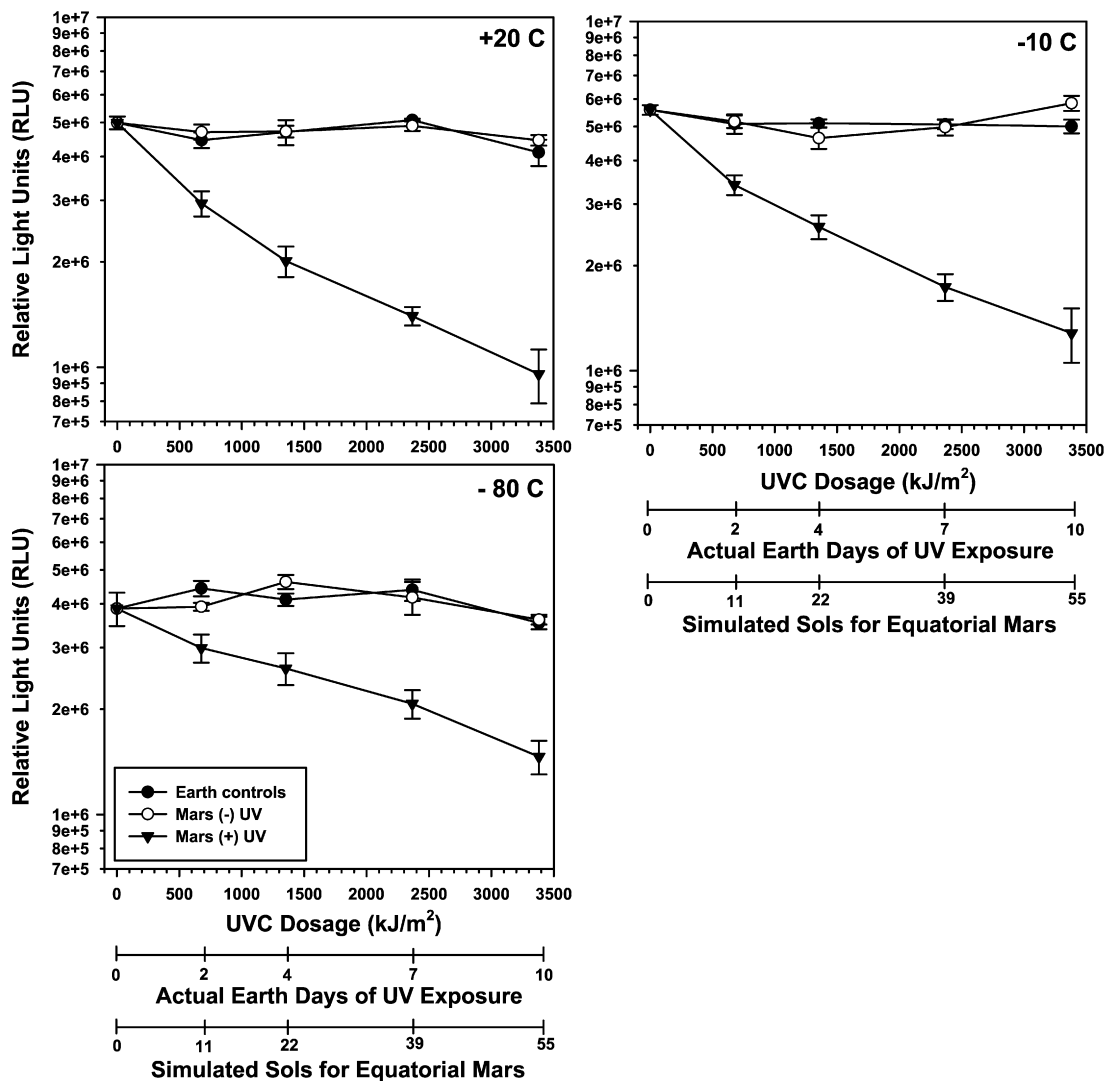


Fig. 2. Adenosine triphosphate (ATP) degradation under simulated martian conditions at +20, -10, or -80 °C. Relative light units (RLU) were derived from the ATP-dependent luciferin/luciferase bioluminescence assay using the BacTiter-Glo Microbial Cell Viability Assay from Promega, Madison, WI, USA. Control treatments for all three temperatures maintained at either Earth-normal or Mars-normal conditions without UV irradiation were similar and did not exhibit any changes over time during the simulations ($P > 0.10$; $n = 6$; bars are standard errors of the means). In contrast, ATP levels on UV-irradiated iridized aluminum coupons decreased steadily over time ($P \leq 0.05$; $n = 6$). Based on the UV models of Moores et al. (2007), an average UVC daily flux at the martian equator equals 65 kJ m^{-2} , and, thus, one Earth day of continuous UV irradiation in the Mars chamber equates to approximately 5.5 sols of UV irradiation on equatorial Mars.

colder temperature of -80 °C was shown to slow down the ATP degradation by a factor of two, relative to the -10 °C treated coupons. In addition, there was a slight increase in the ATP degradation rate at +20 °C, compared to -10 °C. The overall model for the effect of temperature on the half-life of ATP degradation by UVC irradiation under simulated martian conditions is given in Fig. 4. These results suggest that ATP degradation rates should be slower during winter months and at high polar latitudes when compared to summer equatorial locations on Mars.

Moores et al. (2007) have reported on a radiative UV transfer model that accurately predicts UV fluence rates for a diversity of optical depths, latitudinal positions, seasonal changes, and terrain configurations on Mars. Using these models, and the UVC fluence rates for ATP degradation given here, the numbers of sols to accumulate a seven half-life degradation of ATP (i.e.,

a 99% reduction in ATP) were estimated for the upper decks of landers (Fig. 5A) and the undersurfaces of spacecraft overhangs (Fig. 5B). First, for equatorial Mars, the number of sols required to accumulate enough UVC for a 99% reduction in ATP was predicted to range between approximately 158 to 1000 sols under optical depths of 0.2 to 5.0, respectively (Fig. 5A). At 75° N latitude, a location similar to the Phoenix landing site, the number of sols required to reach a similar ATP degradation level would be achieved in approximately 320 to 10,000 sols at optical depths of 0.2 to 5.0, respectively. These results are dramatically longer than originally predicted, and they indicate that ATP is likely to have very long residence times on Mars, even when fully exposed to high solar UVC fluence rates near the equator.

In contrast, if ATP were present on the undersurfaces of spacecraft landed on Mars, the predicted residence times for

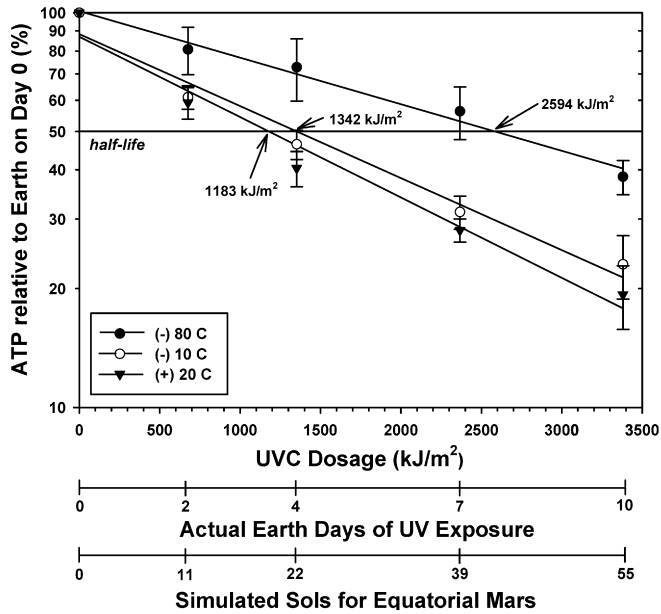


Fig. 3. Regression models for ATP degradation with UV irradiation under martian conditions and incubated at -80 , -10 , and $+20$ °C. All regression models were highly significant ($P \leq 0.0001$) and exhibited linear declines in detected ATP over time. Half-life values were estimated as 2594, 1342, or 1183 kJ m^{-2} for the experiments maintained at -80 , -10 , and $+20$ °C, respectively. Regression models were estimated as: (a) -80 °C, $y = -0.01511x + 96.7$, $r^2 = 0.5309$; (b) -10 °C, $y = -0.0182x + 84.9$, $r^2 = 0.7942$; and (c) $+20$ °C, $y = -0.01898x + 83.4$, $r^2 = 0.7801$ ($n = 6$; bars are standard errors of the means). Based on the UV models of Moores et al. (2007), an average UVC daily flux at the martian equator equals 65 kJ m^{-2} , and, thus, one Earth day of continuous UV irradiation in the Mars chamber equates to approximately 5.5 sols of UV irradiation on equatorial Mars.

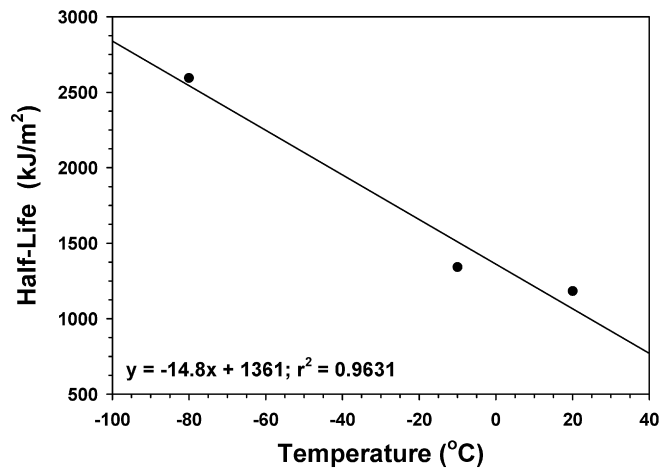


Fig. 4. Linear model for the half-life values versus temperature for the regression models of ATP degradation presented in Fig. 3 ($P \leq 0.0001$).

a 99% reduction in ATP due to UVC degradation alone would be approximately 2 orders of magnitude longer (Fig. 5B). The accumulation of UVC on the undersides of spacecraft is determined exclusively by the upwelling of reflected UVC photons from the surrounding soil and rock terrain. The UV model for spacecraft undersurfaces used here assumed a terrain similar to the MPF landing site extrapolated to a generally flat surface (i.e., we did not assume that the rovers or landers would be dra-

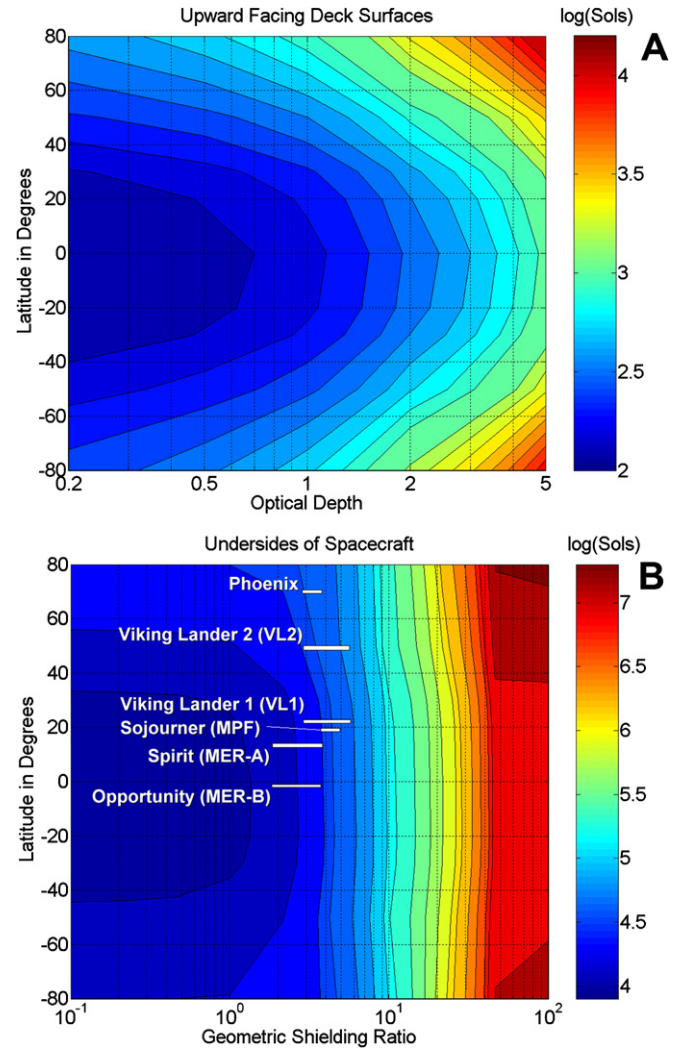


Fig. 5. Results of a one-dimensional radiative transfer model (Moores et al., 2007) for UV flux on Mars developed for a 99% reduction (seven sequential half-lives) in ATP over time. (A) Under clear sky conditions ($\tau = 0.5$), survival times for a reduction of 99% of ATP on horizontal Sun-exposed upper surfaces of spacecraft varied from approximately 158 sols for equatorial locations to 975 sols for polar landing sites. (B) Residence times for a 99% reduction of ATP present on undersurfaces of spacecraft were significantly longer than upper surfaces and were approximately 3.2×10^4 sols for the Viking, Sojourner, Spirit, Opportunity, and Phoenix vehicles.

matically pitched on a sloped surface which might expose the undersurfaces to direct UV irradiation at low Sun angles). In order to estimate the upwelling UVC fluence rates for spacecraft undersurfaces, the UV model used a “geometric shielding ratio” (GSR) to scale the upwelling UVC to a generalized term for the entire undersides of vehicles. The GSR term is defined as the width of a spacecraft undersurface divided by the height above the ground of the underside. The GSR values were then used to scale the upwelling UVC irradiation to predict the number of sols required to achieve a seven half-life reduction in ATP. As GSR increases, the number of sols required to accumulate a given UVC dosage increases, and, thus, the time required to achieve a given level of ATP degradation lengthens. The GSR values for the two Viking landers; the MPF Sojourner, Spirit and Opportunity rovers; and the Phoenix lander fall between 2

and 6, and thus are considered similar for the following predictions. The bars in Fig. 5B for each vehicle represent the range of GSR values on different locations on the undersides of these vehicles. The ends of the bars at the higher GSR values represent the center of the landed vehicles, and the ends of the bars at the lower GSR values represent locations on the undersurfaces near the edges of the vehicles. For simplicity, the overall GSR average of all vehicles was considered to be 4.5, which yields approximately 32,000 sols to accumulate enough UVC irradiation to produce a 99% reduction in ATP. The model in Fig. 5B was generated assuming an optical depth of 0.5, and, thus, the number of sols required under global dust storm conditions would be expected to increase the residence time of ATP on undersurfaces by one to two orders of magnitude.

The numbers of sols predicted above for various spacecraft configurations, assumed that UVC was the sole factor in degrading the ATP on spacecraft surfaces on Mars. This assumption is based on the observation that UVC photons between 200 and 250 nm are largely responsible for the UV degradation of organic molecules (McLauren and Shugar, 1964). However, as other factors are described that can either enhance or retard the degradation of ATP under martian conditions, these estimates must be adjusted. For example, an experiment was designed to determine if different spacecraft materials could enhance or retard the degradation of ATP under simulated martian conditions. Six spacecraft materials were exposed to the standard martian conditions of 4.1 W m^{-2} UVC ($\tau = 0.1$), -10°C , 7.1 mb, and Mars gas mix for 7 Earth-days to determine the effects of the materials on ATP degradation. The standard “control” spacecraft material in the simulations discussed above, and here, was iridized aluminum coupons. Results (Fig. 6) indicated that ATP on astroquartz, graphite, or iridized aluminum degraded at similar rates for the UV-exposed coupons. The survival rates of ATP on these materials exposed to Mars conditions but protected from UVC irradiation were not significantly different from $T = 0$ samples at the start of the experiment. In contrast, ATP doped coupons of clear- and black-anodized aluminum, and stainless steel exhibited significantly faster degradation rates than iridized aluminum. The increased rate of ATP destruction was greatest for stainless steel surfaces, and was over two orders of magnitude greater than that observed for iridized aluminum. What was even more surprising was the observation that the $T = 0$ and Mars controls without UVC samples of clear- and black-anodized aluminum and stainless steel exhibited lower recovered ATP luminescence values than similar iridized aluminum control coupons. These results support the conclusions that (i) ATP may be bound at different rates on different materials, and (ii) ATP is degraded by UVC to a greater extent on anodized aluminum and stainless steel surfaces as compared to iridized aluminum, graphite, and astroquartz. Based on the data in Figs. 5 and 6, ATP on the upper surfaces of Sun-exposed spacecraft components may degrade by 99% in only 1.5 to 2 sols on stainless steel but take as long as 150 to 200 sols on iridized aluminum.

A second materials ATP degradation experiment was conducted in which untreated aluminum and iridized aluminum coupons were either heat-treated or not heat-treated to deter-

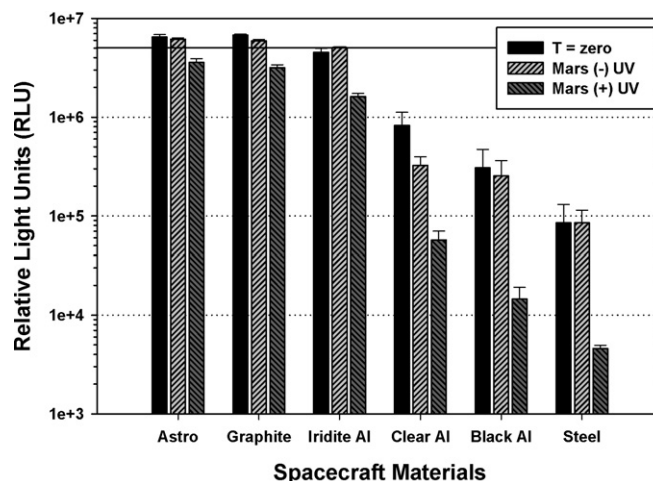


Fig. 6. Effects of ATP degradation on six spacecraft materials at $T = 0$ or under Mars conditions. Astroquartz, graphite, and iridized aluminum coupons with ATP assayed at $T = 0$ or exposed to Mars (–) UV conditions for 7 d were similar and not significantly different from the initial ATP concentration of 5×10^6 relative light units (RLU) in the 50 μl drops added to each coupon (horizontal bold line). However, application of ATP to clear-anodized aluminum, black-anodized aluminum, and stainless steel resulted in 1–2 orders of magnitude decreases in the RLU values on coupons assayed at $T = 0$ or exposed to Mars (–) UV conditions. Following UV irradiation under martian conditions, the recovered ATP levels were affected to a higher degree on the clear-anodized aluminum, black-anodized aluminum, and stainless steel coupons when compared to the astroquartz, graphite, and iridized aluminum coupons ($P \leq 0.05$; $n = 6$; bars are standard errors of the means).

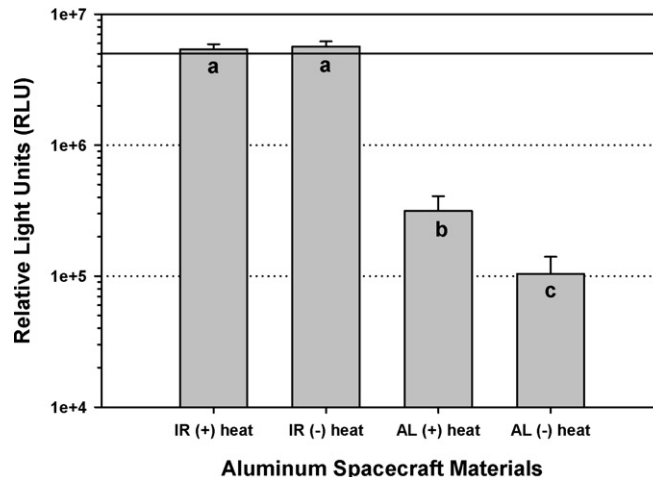


Fig. 7. Effects of ATP degradation on coupons with both uncoated (AL) and iridized (IR) aluminum. Coupons were either heat-treated at 130°C for 24 h [(+) heat] or not heat-treated [(-) heat] prior to doping coupons with ATP. Recovery of ATP from iridized aluminum coupons was similar to the control concentration of 5×10^6 relative light units (RLU) in the 50 μl drops added to each coupon (horizontal bold line). Significantly higher levels of ATP were recovered from iridized coupons than from uncoated coupons, and heating uncoated aluminum coupons increased the recovered ATP levels as compared to the unheated and uncoated aluminum coupons ($P \leq 0.05$; $n = 6$; bars are standard errors of the means).

mine if the coating or heat-sterilization of the materials would affect the rates of ATP recovered. Coupons were not exposed to Mars conditions or UVC irradiation, but were doped with ATP, as described above, and immediately assayed for ATP (Fig. 7). Results indicated that when the aluminum coupons

were treated with the Iridite chromium oxide coating, the ATP was stable, and not affected by heat-sterilization. However, when untreated aluminum coupons were doped with ATP, the recovered amounts of ATP were significantly lower than the iridized surfaces. In addition, heat-sterilization of untreated aluminum coupons slightly increased the level of ATP recovered when compared to the unheated and untreated aluminum coupons. These results support the conclusion that both the surface treatment and the level of heat-sterilization completed prior to spacecraft assembly can alter the recovery rates of ATP from spacecraft surfaces. Reductions in the amounts of ATP recovered from these coupons could be caused by direct metal alterations of the ATP molecules (i.e., degradation), changes in the adhesion of ATP molecules to surfaces of divergent chemistries, or both. These results serve to emphasize that the procedures in which spacecraft materials are handled prior to launch can affect the recovery of ATP, and presumably other biogenic signature molecules, and, thus, more research is required to better characterize these processes.

The ATP molecule is composed of D-ribose, adenine, and three phosphate groups. In order to determine how UVC irradiation in the Mars simulations was degrading ATP, chemical analyses of recovered ATP were conducted using LCMS. Following UVC irradiation under martian conditions, the peak at 252 m/z ($[\text{ATP-2H}]^{2-}$) was significantly reduced, while the peak at 425 m/z ($[\text{ADP-H}]^{-}$) increased sharply while all other peaks remained approximately similar (Fig. 8). The decrease of the 252 peak and increase of the 425 peak are interpreted to mean that the primary effect of UVC irradiation was to remove the γ -phosphate group from ATP with little additional degradation of the resulting adenosine diphosphate (ADP) molecule. No evidence was observed for the degradation of the D-ribose or adenine moieties of ATP or ADP. The removal of the γ -phosphate group from ATP would prevent the luciferin/luciferase bioluminescence assay from detecting ATP.

Liquid chromatography mass spectroscopy was also used to determine if ATP was directly degraded by, or more aggressively adhered to, iridized aluminum or stainless steel without UV exposure. All coupons were doped with 50 picomoles of ATP, air-dried, and stored for 48 h prior to analysis. First, the LCMS spectrum from a water-blank control exhibited very low levels of background noise (Fig. 9A). Second, the recovery efficiency of ATP from iridized aluminum was 95–100% efficient, and LCMS analysis yielded spectra predominated by peaks at 252.4 m/z which corresponded to $[\text{ATP-2H}]^{2-}$ molecules (Fig. 9B). And third, similar analyses for ATP-doped stainless steel yielded LCMS spectra without strong $[\text{ATP-2H}]^{2-}$ peaks (compare Figs. 9B and 9C). No evidence was observed that would indicate that any physical degradation of the ATP molecules occurred during exposure to the two materials. Results support the conclusion that ATP is bound with stronger affinity by stainless steel than by iridized aluminum.

4. Discussion

The luciferin/luciferase bioluminescence assay for ATP has been proposed as a modern molecular tool for monitoring mi-

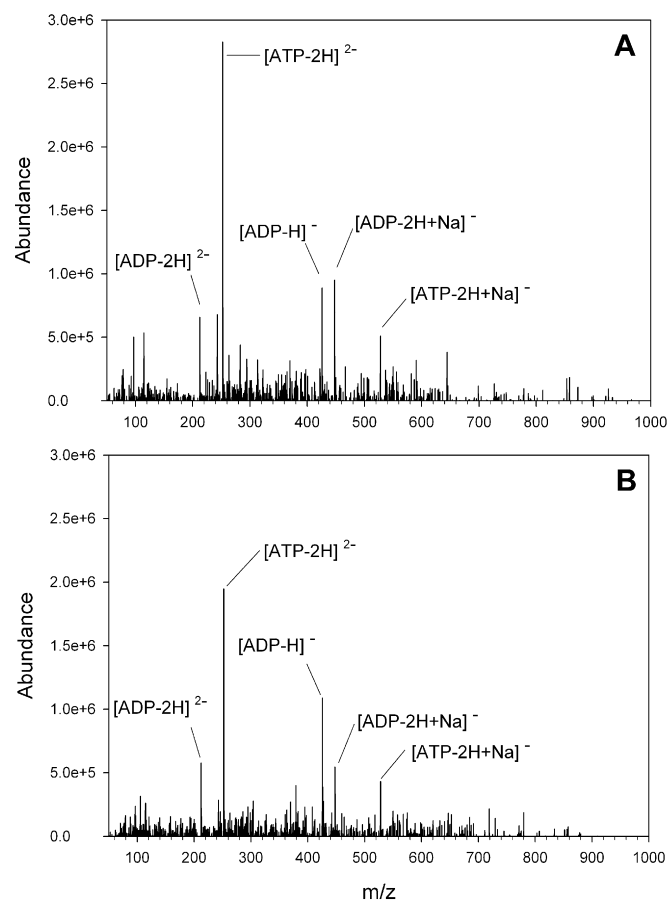


Fig. 8. Liquid chromatography-mass spectrometry (LCMS) spectra of ATP degradation products with and without Mars UV simulation. (A) Peaks indicate the relative abundance of the parent ATP molecule (peak at 252 m/z) plus various breakdown products formed in the LCMS analyses when samples were not exposed to simulated UV irradiation under martian conditions. (B) The $[\text{ATP-2H}]^{2-}$ peak at 252 m/z decreases, and the $[\text{ADP-2H}]^{-}$ peak at 425 m/z increases, after UV irradiation indicating that the γ -phosphate group is the primary degradation product of the ATP molecule under martian conditions. No evidence was observed in the LCMS spectra of UV degradation of the α - or β -phosphate, D-ribose, or adenine moieties of ATP.

crobial contamination on spacecraft surfaces (La Duc et al., 2004; Venkateswaran et al., 2003). The assay is fast, economical, and relatively simple to use. In addition, the luciferase ATP assay has the advantages of detecting both cultivable and noncultivable microbial species, and in detecting extracellular versus intracellular ATP. Although there can be a bias in the assay against the detection of spore-forming species like *Bacillus* and *Clostridium* due to very low concentrations of ATP in bacterial endospores (Setlow and Kronberg, 1970), the ATP assay shows promise for improving the monitoring of microbial contamination of spacecraft prior to launch.

Results from recent studies that used the luciferase ATP assay to monitor spacecraft microbial contamination (La Duc et al., 2004; Venkateswaran et al., 2003) and simulated bioregenerative life support systems (Moissl et al., 2007) indicate that ATP is ubiquitous on spacecraft surfaces and space hardware, and may even remain after some sterilization and cleaning procedures have been completed. Thus, it is likely that intact extracellular ATP molecules will make it to Mars as surface conta-

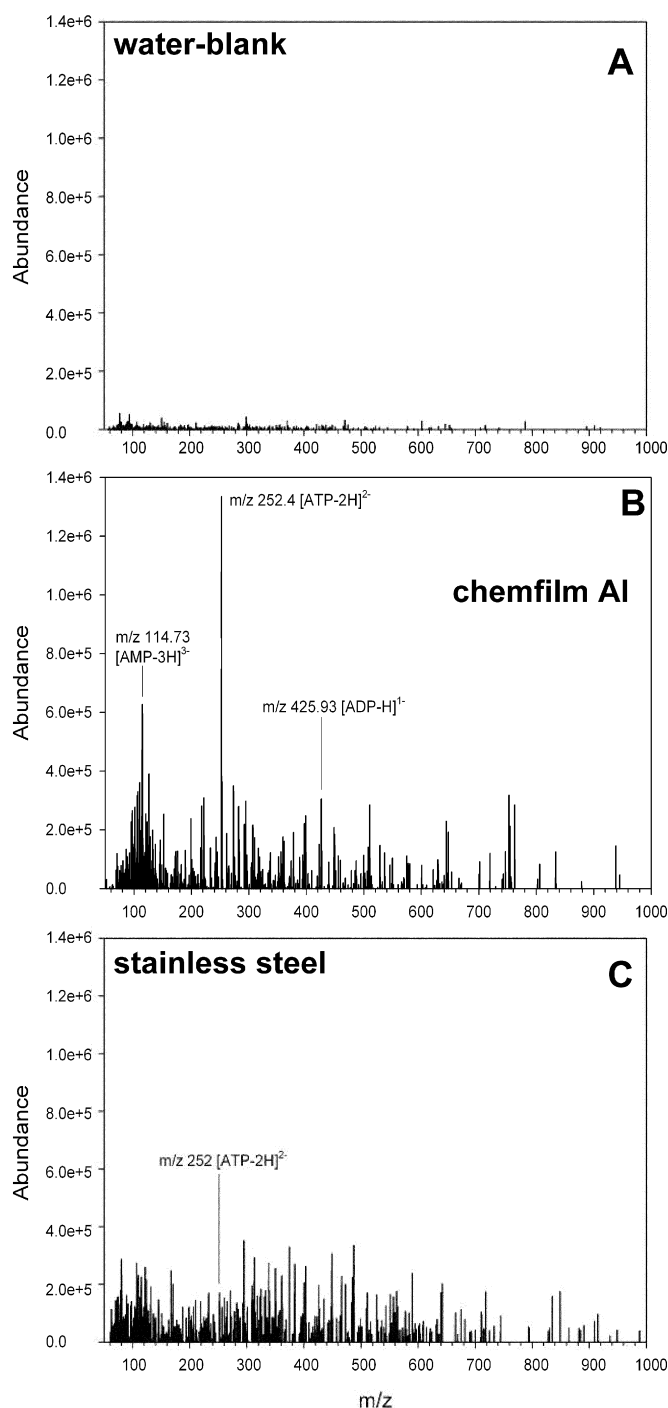


Fig. 9. Liquid chromatography-mass spectrometry (LCMS) spectra of ATP retention on iridized aluminum and stainless steel coupons that were not exposed to UV irradiation or simulated martian conditions. (A) LCMS spectrum of a negative water-blank control sample. (B) LCMS spectrum of ATP eluted from iridized aluminum coupons. (C) LCMS spectrum of ATP eluted from stainless steel coupons.

mination on spacecraft. The presence of extracellular ATP may not be a problem for planetary protection considerations that are concerned about preventing the contamination of the martian surface with viable terrestrial microorganisms, but could pose a risk of generating false positives in future life-detection experiments that rely on detecting organic biosignature mole-

cules. Thus, the fate of extracellular ATP on spacecraft surfaces on Mars must be characterized in order to better design spacecraft cleaning protocols. The current study was constrained to characterizing the UV degradation of extracellular ATP on spacecraft materials because previous research had shown that spores of a number of known spacecraft contaminants are likely to be rapidly killed by UV irradiation after just a few hours on sol 1 after landing (Newcombe et al., 2006; Schuerger et al., 2003, 2006), and biogenic molecules may persist well beyond the loss of microbial viability (Cockell et al., 2005; Tauscher et al., 2006).

Extracellular ATP persisted much longer than previously published kill rates for spore viability (Newcombe et al., 2006; Schuerger et al., 2003, 2006) under simulated martian conditions, including high UVC fluence rates. It was initially anticipated that ATP would exhibit degradation rates under martian conditions somewhat slower than the loss of bacterial spore viability (Schuerger et al., 2003, 2006), but not dramatically slower. In fact, the UV degradation rates for ATP were found to be greater than 10 orders of magnitude slower than loss of spore viability. For example, *Bacillus pumilus* SAFR-032 is a high UVC-resistant bacterial strain recovered from a spacecraft assembly facility at JPL (Link et al., 2004). However, approximately 2×10^6 dormant endospores of *B. pumilus* SAFR-032 per sample were killed within 180 min under an equatorial Mars simulation of 39 kJ m^{-2} of accumulated UVC irradiation (Schuerger et al., 2006). Other *Bacillus* spp. have been shown to be even more sensitive to UVC than *B. pumilus* SAFR-032, and, thus, would be inactivated in much shorter times than 180 min on equatorial Mars (Newcombe et al., 2006; Schuerger et al., 2003, 2006). In contrast, extracellular ATP was found to degrade in the current study by only 50% (i.e., one half-life) during a Mars simulation in which the samples were exposed to 1342 kJ m^{-2} of UVC irradiation (22 simulated days on Mars). These results are consistent with other studies (Stoker and Bullock, 1997; ten Kate et al., 2005, 2006) in which UV-degradation of the amino acid, glycine, was tested under a diversity of martian conditions and found to persist for reasonably long periods of times. In two separate papers, ten Kate et al. (2005, 2006) reported half-lives for glycine of 22 and 250 h; and Stoker and Bullock (1997) reported significant levels of glycine were still detectable in a Mars UV and atmosphere simulation after 18 d. Furthermore, other biogenic signature molecules (e.g., phycobilisomes, esterases, DNA, and chlorophyll) have been shown to persist for up to 15–20 times longer than cell viability in *Chroococcidiopsis* sp. 029 exposed to martian conditions (Cockell et al., 2005). These results are all consistent with the conclusion that spores of terrestrial microorganisms under Mars surface conditions will be inactivated very quickly if exposed to solar UV irradiation, but that several common biogenic signature molecules are likely to persist for considerably longer. Thus, from a planetary protection perspective, long-term survival of biogenic organic molecules on spacecraft surfaces may constitute a much greater risk for the forward contamination of science payloads than the persistence of viable bioloads on vehicles.

Although ATP degradation under high UVC irradiation was affected by temperature, the biogenic signature molecule would still be expected to persist on spacecraft surfaces on Mars for years and perhaps decades. In the current study, ATP degradation by UVC irradiation was nearly double the rate at 20 °C when compared to the lowest temperature tested, −80 °C. In their work with the UV degradation of glycine, [ten Kate et al. \(2006\)](#) also reported a strong effect of temperature under martian conditions showing that the rate of UV degradation of glycine was nearly one order of magnitude faster at 21 °C compared to −63 °C. These results are explicable by the simple principle of chemical reaction rates increasing with higher temperatures. In addition, these results suggest that the UV-induced degradation of biogenic molecules will be moderately suppressed at high polar latitudes and seasonal changes in which the surface temperatures on spacecraft are significantly below 0 °C.

The half-life of ATP under the martian conditions tested here at −10 °C was found to be 1342 kJ m^{−2} (i.e., 22 simulated sols on Mars) at a UVC flux of 4.1 W m^{−2}. Using the recently published Mars UV radiative transfer models of [Moore et al. \(2007\)](#), the long-term persistence of ATP on iridized aluminum was estimated for the upward facing Sun-exposed surfaces and shaded undersides of spacecraft. Results indicate that a 99% reduction of ATP (i.e., seven half-lives) would be achieved on upward facing Sun-exposed surfaces within approximately 158 sols on equatorial Mars under an optical depth of 0.5, and perhaps as long as 1000 sols under global dust storm conditions of optical depths near 5. In contrast, ATP would likely persist for up to 32,000 sols if present as surface residues on the undersides of vehicles (assuming a GSR value of 4.5 for all vehicles and UV as the only degrading factor). These long residence times suggest that some biogenic molecules might be detected on spacecraft surfaces for the nominal lengths of most missions.

However, several other factors on the martian surface are likely to enhance the destruction of ATP, and, thus, the UV degradation rates of ATP discussed above should not be used to estimate the actual residence times on spacecraft surfaces on Mars until other factors that contribute to ATP destruction are described. For example, data presented herein suggests that the types of spacecraft materials may affect the UV degradation of ATP under martian conditions. In addition, in a recent paper, [Schuerger et al. \(2006\)](#) listed 13 biocidal factors that might contribute to the inactivation of viable spacecraft bioloads on Mars including (not in priority): (1) solar UV irradiation, (2) low pressure, (3) extreme desiccating conditions, (4) extreme diurnal temperature fluctuations, (5) solar particle events, (6) galactic cosmic rays, (7) UV-glow discharge from blowing dust, (8) solar UV-induced volatile oxidants [e.g., O₂, O[−], H₂O₂, NO_x, O₃], (9) globally distributed oxidizing soils, (10) extremely high salt levels [e.g., MgCl₂, NaCl, FeSO₄, and MgSO₄] in surficial soils at some sites on Mars, (11) high concentrations of heavy metals in martian soils, (12) likely acidic conditions in martian regolith, and (13) high CO₂ concentrations in the global atmosphere. Of these biocidal factors, (a) UV photons from solar irradiation and glow discharges from blow-

ing dust, (b) solar UV-induced volatile oxidants, and (c) globally distributed oxidizing soils are likely to be cofactors in the degradation of organics on Mars.

First, spacecraft materials and coatings may enhance the UV-induced degradation of ATP under martian conditions. We report here for the first time that the types of spacecraft materials can have dramatic effects on the rates of ATP destruction under martian conditions. In the current study, ATP was more aggressively destroyed by UVC on clear- and black-anodized aluminum, and stainless steel coupons, as compared to astroquartz, graphite and iridized aluminum ([Fig. 6](#)). On stainless steel, the UV-induced destruction of ATP was enhanced by more than 2 orders of magnitude relative to the iridized aluminum coupons. Thus, if ATP were present on stainless steel surfaces, versus iridized aluminum, the actual UV-induced degradation rates might be as short as 1.5 sols on Sun-exposed upper-decks of vehicles, and no longer than 320 sols on the shaded undersides of rovers or landers. In a separate experiment, we observed that ATP was retained at different rates on aluminum coupons depending on whether the aluminum was coated with Iridite and/or heat-sterilized ([Fig. 7](#)). In addition, LCMS data supports the conclusion that some ATP was preferentially retained on stainless steel surfaces not exposed to UV irradiation ([Fig. 9](#)), possibly indicating that the biogenic molecule might adhere irreversibly to some metal surfaces (stainless steel) and not others (Iridite aluminum). Thus, it appears that ATP can differentially adhere to different materials, and that these same materials might then have divergent effects on UV-induced destruction of ATP under martian conditions. However, care must be taken to also consider that other factors besides the base materials themselves could be involved with ATP retention or degradation under martian conditions. For example, although the coupons in the current study were treated with acid washes prior to use, organic or inorganic residues on spacecraft materials may also affect the retention or degradation processes of ATP on Mars. One application of the chemical properties of spacecraft components might be the prelaunch selection of materials that will promote the destruction of terrestrial biogenic molecules on spacecraft surfaces when such degradation is desirable (e.g., digging and drilling appendages on rovers); and retard the destruction of in situ organics when such destruction would alter the performance of scientific payloads (e.g., sample holding devices within an organic detection payload).

Second, UV photons may be released from blowing dusts on Mars as glow discharges ([Mills, 1977; Buhler and Calle, 2003](#)) at high enough flux densities to contribute to the destruction of organic molecules on Mars ([Calle et al., 2006](#)). The UV photons in glow discharges are released as either vacuum UV (<200 nm) or as short wavelength UVC (200–240 nm) irradiation. Thus, an additional source for UV photons might be present on Mars besides solar irradiation that could contribute to the overall UV environment, and enhance the UV-induced destruction of biogenic molecules. If confirmed, glow discharge UV irradiation could extend the times for UV-destruction of organic molecules on spacecraft to nighttime hours on Mars, and may be present even during global dust storms.

And third, the destruction of ATP under actual martian conditions could be enhanced by the putative presence of strong volatile oxidants near the surface (Bullock et al., 1994; Hunt, 1979; Yen et al., 2000; Zent and McKay, 1994). In the current study, volatile oxidants were not measured, but were likely not produced in quantity. We base this supposition on two studies (Yen et al., 2000; Zent, 1998) in which oxidants were only produced when UV irradiation under Mars conditions interacted with simulated martian soils that contained bound H₂O. Furthermore, Stoker and Bullock (1997) demonstrated that the UV-destruction of glycine was dramatically enhanced by mixing the glycine into a simulated Mars regolith (a Hawaiian palagonite) as compared to glycine deposited on silica glass coupons. Thus, because no simulated Mars soils were included in the current study, it is likely that ATP destruction rates would be enhanced on the surface of Mars by the presence of volatile oxidants generated under in situ conditions. Furthermore, volatile oxidants have likely activated the martian regolith through diffusion (Yen et al., 2000; Zent and McKay, 1994), and may contribute to the destruction of organics by direct contact. Thus, if martian dusts or soils are deposited on the upper decks of vehicles during either landing or operational activities, some terrestrial organics may be partially degraded by the oxidizing soils. In addition, diurnal changes in UV flux may create diurnal changes in volatile oxidants, and, thus, a day/night cycle could alter the actual degradation rates of organics on Mars. Ongoing experiments are currently exploring the effects of Mars analog soils and diurnal cycles on the destruction of ATP and other organics under martian conditions.

5. Conclusions

A robust Mars simulator has been developed for the study of the survival, growth, and evolution of terrestrial microorganisms under Mars conditions, and for the study of the UV degradation of biogenic signature molecules. The Mars Simulation Chamber can accurately simulate five key parameters of the martian surface including high UV flux, low pressure, low temperature, gas composition, and atmospheric dust loading. The Mars chamber was used to study the UV degradation of the biogenic signature molecule, adenosine triphosphate (ATP), in order to characterize the residence times of ATP under a diversity of martian conditions. Results support the conclusion that ATP is likely to survive many orders of magnitude longer than cell or spore viability under Mars UV fluence rates. Survival of ATP on Mars may extend from years to decades on some materials like astroquartz, graphite, and iridized aluminum. In contrast, other materials like stainless steel and anodized aluminum may enhance degradation of ATP shortening residence times to a few sols on upper-deck Sun-exposed surfaces and approximately 320 sols for the undersides of vehicles. However, predicting the actual times on Mars in which ATP might be detectable on spacecraft will depend on the levels of prelaunch spacecraft sterilization and cleaning activities, and on modeling other factors besides UV irradiation

that might contribute to the destruction of organic molecules on Mars.

Based on the literature discussed above and on the results of the current study, we propose that cell/spore viability of landed bioloads should be thought of as sensitive to the martian environment exhibiting rapid inactivation within a few hours on sol 1 after landing, and the survival times of biogenic signature molecules should be viewed as resistant to martian conditions and may persist for years and perhaps decades on certain surfaces of spacecraft. If confirmed, there are three key consequences of these results. First, prelaunch sterilization and cleaning protocols for organics on vehicles with life-detection or organic characterization payloads will likely be required to undergo additional scrutiny in order to lower the diversity and concentrations of residues. However, if mission science objectives are not directly impaired by the presence of biogenic molecules (e.g., a pure Mars geology mission), then organic contamination of spacecraft surfaces may not constitute a significant planetary protection risk. Second, UV irradiation alone does not appear capable of rapid destruction of organic molecules on Mars, and, thus, in situ organics may be more easily detected if present in the shallow subsurface when lander digging operations expose the organics for short periods of time. For example, the Phoenix lander will be launched in August 2007 to search for buried organics in the top 0.5 m of the surface at a high northern latitude. If the Phoenix digging operations expose buried in situ organics, the results from the current study suggest that the putative organics in the trench might be stable for at least several sols to perhaps even several weeks under the solar UV irradiation environment within the trench. And third, when modeling possible effects of large human bases on the surface of Mars, considerations must be given to the possibility that outgassing or dispersal of terrestrial organics from the habitats to the surrounding terrain may constitute a greater forward contamination risk than the dispersal of viable bioloads. Clearly, more study is required to further characterize the rates of UV-induced destruction of terrestrial organic molecules on Mars.

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