

**Submitted to the journal Astrobiology.**

**Title:**

A New Analysis of Mars 'Special Regions': Findings of the Second MEPAG  
Special Regions Science Analysis Group (SR-SAG2)

**Full names and institutional affiliations of all authors:**

John D. Rummel<sup>1</sup>, David W. Beaty<sup>2</sup>, Melissa A. Jones<sup>2</sup>, Corien Bakermans<sup>3</sup>,  
Nadine G. Barlow<sup>4</sup>, Penny Boston<sup>5</sup>, Vincent Chevrier<sup>6</sup>, Benton Clark<sup>7</sup>, Jean-  
Pierre de Vera<sup>8</sup>, Raina V. Gough<sup>9</sup>, John E. Hallsworth<sup>10</sup>, James W. Head<sup>11</sup>,  
Victoria J. Hipkin<sup>12</sup>, Thomas L. Kieft<sup>5</sup>, Alfred S. McEwen<sup>13</sup>, Michael T.  
Mellon<sup>14</sup>, Jill Mikucki<sup>15</sup>, Wayne L. Nicholson<sup>16</sup>, Christopher R. Omelon<sup>17</sup>,  
Ronald Peterson<sup>18</sup>, Eric Roden<sup>19</sup>, Barbara Sherwood Lollar<sup>20</sup>, Kenneth L.  
Tanaka<sup>21</sup>, Donna Viola<sup>13</sup>, and James J. Wray<sup>22</sup>

<sup>1</sup>East Carolina University, Greenville, NC 27858, USA, <sup>2</sup>Jet Propulsion

Laboratory, California Institute of Technology, Pasadena, CA 91109, USA,

<sup>3</sup>Altoona College, Pennsylvania State University, Altoona, PA 16601, USA,

<sup>4</sup>Department of Physics and Astronomy, Northern Arizona University, Flagstaff,

AZ 86011, USA, <sup>5</sup>New Mexico Tech, Socorro, NM 87801, USA, <sup>6</sup>University of

Arkansas, Fayetteville, AR 72701, <sup>7</sup>Space Science Institute, Boulder, CO 80301, USA, <sup>8</sup>German Aerospace Center, Institute of Planetary Research, D-12489 Berlin, Germany, <sup>9</sup>Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA <sup>10</sup>Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast BT7 9BL, UK, <sup>11</sup>Brown University, Providence, RI 02912, USA, <sup>12</sup>Canadian Space Agency, Saint-Hubert, QC J3Y 8Y9, Canada, <sup>13</sup>University of Arizona, Tucson, AZ 85721, USA, <sup>14</sup>Southwest Research Institute, Boulder, CO 80301, USA, <sup>15</sup>University of Tennessee, Knoxville, TN 37996, USA, <sup>16</sup>Department of Microbiology and Cell Science, University of Florida, Merritt Island, FL 32953, USA, <sup>17</sup>Department of Geological Sciences, The University of Texas at Austin, Austin, TX 78712, USA, <sup>18</sup>Queen's University, Kingston, ON K7L 3N6, Canada, <sup>19</sup>University of Wisconsin, Madison, WI 53706, USA, <sup>20</sup>University of Toronto, ON M5S 3B1, Canada, <sup>21</sup>US Geological Survey, Flagstaff, AZ 86001, USA, <sup>22</sup>School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA.

**Corresponding Author:**

Dr. John D. Rummel

Institutional Address: Department of Biology, c/o ICSP, Flanagan 250, East  
Carolina University, Greenville, NC 27858, USA

Current Mailing Address: P.O. Box 2838, Champlain, NY 12919, USA

Telephone: 1-508-523-1317 (cell); 1-252-328-1755 (desk); Fax: 1-252-328-4265

E-mail: <rummelj@ecu.edu>

**Running title:**

Mars Special Regions Update (SR-SAG2)

**Abstract**

A committee of the Mars Exploration Planning and Analysis Group (MEPAG) has reviewed and updated the description of Special Regions on Mars as places where Earth organisms might replicate (per the COSPAR Planetary Protection Policy. This review and update was conducted by an international team (SR-SAG2) drawn from both the biological science and Mars exploration communities, focused on understanding when and where Special Regions could occur. The study applied recently available data about Mars environments and about Earth organisms, building on a previous analysis of Mars Special Regions

(2006) undertaken by a similar team. Since then, a new body of highly relevant information has been generated from the Mars Reconnaissance Orbiter (launched 2005), Phoenix (2007), and data from Mars Express and the twin MER landers (all 2003). Results have also been gleaned from the Mars Science Laboratory (launched 2011). In addition to Mars data, there is a considerable body of new data regarding the known environmental limits to life on Earth—including the potential for terrestrial microbial life to survive and replicate under martian environmental conditions. The SR-SAG2 analysis has also included an examination of new models of Mars relevant to natural environmental variation in water activity and temperature, a review and reconsideration of the current parameters used to define Special Regions; and updated maps and descriptions of the Mars environments that are recommended for treatment as “Uncertain,” or “Special,” as natural features or those that could be formed by the influence of future landed spacecraft. Several significant changes in our knowledge of the capabilities of Earth organisms and the existence of possibly habitable Mars environments have led to a new appreciation of the where Mars Special Regions may be identified and protected. The SR-SAG has also considered the impact of Special Regions on potential future human missions to Mars, both as the locations of future resources and as places that should not be inadvertently contaminated by human activity.

**Key Words or Phrases:**

Mars environments, Mars astrobiology, extreme environment microbiology, planetary protection, exploration resources

**1. Introduction**

Since the beginning of human activity in space science and exploration, there has been an appreciation of the potential negative outcomes of transferring life from one planet to another. Given the unknown consequences of contact between two biospheres and the fundamental value of studying a possible new life-form in isolation from Earth life, thoughtfulness and caution are warranted. Those ideas are reflected in both the United Nations (UN) Space Treaty of 1967 (United Nations, 1967) and in the International Council for Science's Committee on Space Research (COSPAR) Planetary Protection Policy (COSPAR, 2011), which serves under the UN treaty as a consensus standard for avoiding harmful biological contamination. The "Special Regions" concept is a component of the COSPAR Planetary Protection Policy for Mars that was derived in 2002 (Rummel *et al.*, 2002). Special Regions are regions "within which terrestrial organisms are likely to replicate" as well as "any region which is interpreted to have a high potential for the existence of extant martian life." Robotic missions planning to have direct contact with such Special Regions are given planetary protection categorization IVc, with stringent cleanliness constraints on the portions of the mission that

could contact such regions. The avoidance of the contamination of Special Regions is also the focus of the “Principles and Guidelines for Human Missions to Mars” (COSPAR, 2011) that are also part of COSPAR’s current policy.

While the original COSPAR definition of “Special Regions” (Rummel *et al.*, 2002) conveyed the concept in qualitative terms, its proposed translation into (mostly) quantitative terms was accomplished by a two-step process that occurred over the course of 2005-2008. The first step was preparation of a technical analysis by a MEPAG (Mars Exploration Program Analysis Group) Special Region Science Analysis Group (SR-SAG; Beaty *et al.*, 2006); this analysis was carried out in 2005-06, with most of the technical information being of early-2006 vintage. The second step involved COSPAR’s development of policy in response to that report. This two-step process resulted in the acceptance (by COSPAR’s Bureau and Council) of the current Special Region definition by COSPAR at the Montreal Assembly in July 2008. COSPAR additionally recommended (Kminek *et al.*, 2010) that the quantitative definitions of Special Regions be reviewed on a 2-year cycle. This study is the first such review since the 2008 definitions were adopted.

There were two major reasons for undertaking the review at this time: 1) It is timely in that both European Space Agency (ESA) and National Aeronautics and Space Administration (NASA) are planning on landed robotic missions to Mars in 2016, as well as follow-on landers in 2018 and 2020 (proposed), and 2) Important

new data sets are now available that have a bearing on the potential locations and nature of Mars Special Regions, which can be included in our considerations. MEPAG's 2006 analysis was based on results from Viking, Mars Global Surveyor (MGS), and initial results from Odyssey (ODY, launched in 2001), Mars Express (MEX, launched in 2003), and Mars Exploration Rovers (MER, launched in 2003). Now, however, a new body of highly relevant data about Mars exists from both the on-going surveys of ODY, MEX, and MER—spacecraft which are still active as of mid-2014—as well as extensive data from the Mars Reconnaissance Orbiter (MRO, launched in 2005), the Phoenix mission (PHX, launched in 2007), and initial results from the Mars Science Laboratory (MSL, launched in 2011). In addition, valuable research has been conducted since 2006 from ground-based, laboratory, analogue, and International Space Station (ISS) studies.

### **1.1. Terminology and Definitions**

The terminology adopted for this study was intended to be consistent with the original MEPAG SR-SAG study (Beaty et al., 2006). Accordingly, the following words are intended to have the same meaning as before:

1.1.1. *Propagate* means to reproduce via cell division, generally accompanied by a biomass increase. Other kinds of activity, including cell maintenance, thickening of cell walls (as one aspect of growth), and mechanical dispersal by aeolian processes are not sufficient to indicate propagation.

1.1.2. *Special Regions*. COSPAR defines Special Regions as “a region within which terrestrial organisms are likely to replicate” and states that “any region which is interpreted to have a high potential for the existence of extant martian life forms is also defined as a Special Region” (COSPAR, 2011). At present there are no Special Regions defined by the existence of extant martian life, and this study concentrates only on the first aspect of the definition.

1.1.3. *Non-Special Regions*. A martian region may be categorized as Non-Special if the temperature and water availability will remain outside of the threshold parameters posited in this study for the time-period discussed below (see 1.4. Constraints). All other regions of Mars are designated as either Special or Uncertain.

1.1.4. *Uncertain Regions*. If a martian environment can simultaneously demonstrate the temperature and water availability conditions identified in this study, propagation may be possible and those regions would be identified as Special Regions. Nonetheless, because of the limited nature of the data available for regions only sensed remotely, it may not be possible to prove that such environments are capable of supporting microbial growth. Such areas are therefore treated in the same manner as Special Regions until they are shown to be otherwise.

1.1.5. *Spacecraft-Induced Special Regions*. Whereas Special Regions may be formed naturally and exist in a natural setting on Mars, even in an otherwise Non-

Special Region a spacecraft may create a non-natural environment that meets the definition of a Special or Uncertain Region, as described above.

[Figure 1 about here]

Fig. 1 shows a Venn diagram picturing the concept of Mars Special Regions addressed in this study, including those that occur naturally and those that may be Spacecraft-induced.

## **1.2. History**

The original MEPAG SR-SAG committee (Beatty *et al.*, 2006) was asked to propose a technical definition of Special Regions, and to evaluate how that definition would apply to Mars. That study focused on the limits to microbial life, and the potential for biologically available liquid water on Mars. The present study has concerned itself with those as well. In that original study the definition of Special Region was determined by a lower temperature limit for propagation (which was given as  $-20^{\circ}\text{C}$ , including margin) and a lower limit for water activity (with margin, an activity threshold of 0.5). In addition, a number of remotely sensed features on Mars were included as Uncertain: recent gullies and gully forming regions; “pasted-on” mantle; low-latitude slope streaks; low-latitude features hypothesized to be glaciers; and features hypothesized to be massive subsurface ice; were considered potentially Special Regions, and if they occur in the future, volcanic environments young enough to retain heat, impact environments young enough and large enough to retain heat, and modern outflow

channels would also be considered Special. In that study, Spacecraft-Induced Special Regions were to be considered on a case-by-case basis with regard to their achieving the temperature and water availability characteristics of Special Regions.

Subsequent to the MEPAG SR-SAG report, which identified sufficient data to distinguish between Special and Non-special Regions using the quantitative parameters of temperature and ( $a_w$ ) to define such regions, the report was referred to the COSPAR Panel on Planetary Protection, which held a Mars Special Regions Colloquium (Kminek *et al.*, 2010) that used that report as the basis to arrive at a consolidated definition for Mars Special Regions for consideration by the COSPAR Planetary Protection Panel, and subsequently by the COSPAR Bureau and Council for inclusion in the COSPAR Planetary Protection Policy.

The COSPAR Colloquium recommended that Special Regions be determined by a lower temperature limit for propagation of  $-25^{\circ}\text{C}$ , which included additional margin and thus was slightly more conservative than the MEPAG SR-SAG limit, and by an identical water activity threshold of 0.5. Building on the MEPAG report, the Colloquium included “dark streaks” of all kinds as features that should be examined on a case-by-case basis to determine if they comprise Special Regions, or Uncertain Regions. Subsequently, the recommendations derived in the COSPAR Colloquium were forwarded to the COSPAR Panel on Planetary Protection and considered at the Montréal Assembly in 2008, and adopted into the

COSPAR policy at that time. Since 2008, the COSPAR definition has been considered as authoritative by both NASA and ESA in their considerations of Mars landing sites (and the preparation of spacecraft landing there), and presumably will be taken up by others when (and if) Mars landings are planned by other nations.

### **1.3. Objectives and Approach for this Study**

The study reported here was guided by a Charter approved by MEPAG in October 2013 and given in Appendix I. As in the original two-step process, it is expected that the results of this MEPAG technical analysis will be reviewed by COSPAR in an international forum and be considered for the furtherance of COSPAR's Planetary Protection Policy regarding Mars. This study, however, was already supplemented by the results of a COSPAR workshop held in April 2014 (Hipkin *et al.*, in preparation) that considered the issues associated with Mars Special Regions and their application, and which brought into play additional non-MEPAG individuals who contributed novel information and perspectives, and thereby contributed directly to the study reported here. Upon the completion of this report, COSPAR will further consider its recommendations and those of the COSPAR workshop, and potentially have one or more additional meetings to aid in the formulation of a recommendation on the extension of the definition of Mars Special Regions that this report includes. That

recommendation will eventually (likely in 2016) be considered by the COSPAR Panel on Planetary Protection and the COSPAR Bureau and Council.

Under the study Charter, this study based its focus about Special Regions on new data available regarding the propagation limits for microbial life, and new data about water on Mars.

1.3.1. *Limits to microbial life and physical conditions on Mars.* The review has considered new low temperature and water (liquid or vapor) utilization limits to microbial growth, including temporary/periodic exposure. The review has also considered new surface and diurnal radiation data from MSL Radiation Assessment Detector (RAD), new diurnal temperature and humidity data from PHX and MSL, and new ISS and analogue chamber experiment results.

1.3.2. *Water on Mars.* Phoenix data have raised interest in perchlorate and other salts as sources of ions that can lower the freezing point of aqueous solutions, as well as participate in their absorbance and deliquescence (the latter particularly with reference to transport issues during roving, drilling, sample collection, and potentially in relation to spacecraft-induced habitable environments). Phoenix also directly excavated both pore-filling and excess ground ice. MRO's Context Imager, High Resolution Imaging Science Experiment (HiRISE), and Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) instruments have presented new evidence of extensive sub polar ground ice, as seen in recent (small) craters. MRO HiRISE also has detected

seasonal Recurring Slope Lineae (RSL, Section 4.1), whereas MRO SHARAD has detected ice cores within lobate debris aprons (LDA). Mars Express data and ground-based astronomy studies have claimed discovery of methane in the martian atmosphere (Formisano *et al.*, 2004; Mumma *et al.*, 2009), for which most potential production mechanisms would infer a co-located liquid water source. While no methane was detected in atmospheric samples by MSL using mass spectrometric or infrared analyses (Webster *et al.*, 2013), there is nonetheless an ongoing debate about the presence and potential fate of methane in the martian atmosphere and the potential for seasonality also exists (cf., Mumma *et al.*, 2009).

The study results reported here have benefited from MSL assessments of past and present habitability at Gale Crater, which were not available to the previous effort. In addition, this study has had a goal to provide information important to the future needs of human explorers on Mars, identifying both the opportunities and cautions regarding Special Regions. Because the SR-SAG2 study has provided an updated list and inventory of features related to the presence of liquid water and other aspects of potentially habitable environments, NASA asked that MEPAG evaluate the relationship between Special Regions and the potential location of and access to resources on Mars of interest to the future human exploration program. Clearly, this linkage between the robotic and human exploration of Mars will grow in significance as the choices implicit in making

Mars a destination (and possible home) for human explorers become more specific.

The MEPAG SR-SAG2 was convened and held its first telecon in November, 2013, but almost all of the technical analysis was carried out during the first 5 months of 2014 (technical data should be judged to be current as of that time). Most of the team's technical exchanges were carried out by e-mail and telecon, though the team additionally made use of a single face-to-face meeting in Boulder, Colorado in January 2014. The team considers itself lucky in a human sense: during the course of this study, the members of the team (see Appendix II) collectively shared the following good news: 1 birth, 2 retirements, and 1 wedding (between 2 of the participants).

#### **1.4 Altered Constraints and Assumptions for this Study**

In addition to new data that might impinge on our understanding of Special Regions, some of the assumptions and constraints used in identifying potential Special Regions have been updated since the previous MEPAG study.

1.4.1 *Depth*. For the 2006 process, the definition of a Special Region was limited to the surface and 5m below because we had almost no observational data below that level, the models were of uncertain quality (but suggested that 5m was below the depth of any seasonal warming affecting subsurface conditions), and it was estimated that impacting spacecraft would not go below 5m depth. For this review we reviewed both depth and temperature, specifically. The review of

depth is based on new thermal modeling, new MRO/MEX radar data, and reported detection of Precambrian water pockets in the Earth's crust (Holland *et al.*, 2013).

1.4.2. *Future Conditions: 500 years.* Whereas in the previous MEPAG study the timescale used to scale the prediction of future conditions was 100 years, the COSPAR Colloquium (Kminek *et al.*, 2010) chose a timescale of 500 years to constrain predictions of geological events that could affect the environmental conditions on Mars. In this study we have concurred with COSPAR, and also specify 500 years as the period over which we can predict that Mars conditions (as they are known today) will not change significantly.

The orbit of Mars is understood to experience large oscillations resulting from periodic forcing from the Sun and neighboring planets (e.g., Ward, 1974). These oscillations most notably occur in the obliquity (tilt of the spin axis), eccentricity, and  $L_s$  (season) of perihelion with an overlap of those periodic forcings taking place between  $10^4$  and  $10^6$  years apart. The result can have a pronounced influence on the global climate including ground temperatures and near-surface water-ice (e.g., Toon *et al.*, 1980; Paige, 1992; Mellon and Jakosky, 1995). For example, an increase in obliquity will shift the deposition of solar energy from equatorial regions toward polar regions and result in a similar shift in ground temperatures. Likewise increased heating in the polar regions will raise summer

sublimation of the polar ice cap and increase atmospheric humidity (Haberle and Jakosky, 1990; Jakosky *et al.*, 1993).

Laskar (2004) provided an integration of solar system dynamics from which these effects may be examined over the next 500 years. Fig. 2 shows a result from this integration; the magnitude of the shift from the present day orbit is small over this relatively short time frame.

[Figure 2 about here]

The resulting effects on ground temperatures, atmospheric humidity, and the distribution of ground ice are expected to be similarly small. Figs. 3 and 4 illustrate the expected changes in annual-mean ground surface temperatures based on a standard Mars thermal model (Mellon and Jakosky, 1992; Mellon *et al.*, 2004). Over the next 500 years, changes in the mean temperatures are between 0-0.2 K and in the maximum temperature changes are less than 0.8 K. These differences are imperceptibly small and generally less than the uncertainties in such ground temperature models.

[Figure 3 about here]

[Figure 4 about here]

In the modern climate, summer-time sublimation of water ice from the polar caps is the primary control on the global atmospheric humidity, with a smaller component of seasonal exchange with the regolith (e.g., Jakosky, 1985). In the next 500 years, there is expected to be a slight increase in polar insolation (Fig. 4),

which may increase the polar-summer sublimation rate by at most a few percent (see Jakosky *et al.*, 1993, Fig. 2). If it is assumed that the polar sublimation rate is linearly proportional to atmospheric water content (for at least small changes) then in 500 years we can expect a similar increase in atmospheric water content.

Ground ice is stable at locations where the annual-mean water-vapor density with respect to ice in the soil pore-space equals that of the atmosphere (e.g., Mellon and Jakosky, 1993). From the changes discussed in ground temperatures and atmospheric humidity in the next 500 yrs, we might expect the depth of ground-ice stability to shift by at most a few % from its current depth. Likewise the geographic equatorward limit of ground-ice stability may shift by less than a degree in latitude, if at all.

The current ground-ice distribution appears to be in equilibrium with an atmosphere containing about 20 precipitable micrometers (pr  $\mu\text{m}$ ) of vertically well-mixed water vapor (Mellon *et al.*, 2004). These forecast changes in temperature and humidity are much smaller than the current uncertainty in ice-stability models and spacecraft data interpretation. Likewise, ground temperature changes will be much too small to result in any melting of pure ice. Melting of a frozen brine may occur only if the conditions are already very close to the eutectic.

It should be noted that in picking this time-period of 500 years within which current conditions regarding Special Regions can be reasonably anticipated to

continue, we are not saying anything regarding the length of time within which we are interested in protecting those Special Regions nor are we making any guesses regarding the number of missions that are expected to land on Mars in that time-period. The 500-year value specified here is *not* related to any “period of biological exploration” that may once have been specified by COSPAR’s Planetary Protection Policy.

[Finding 1-1 here]

## **2. Life on Earth: General Considerations Regarding Its Propagation on Mars**

### **2.1 Introduction to Earth organisms: Chemolithoautotrophs**

It is reasonable to consider what types, or categories, of terrestrial organisms that could have the potential to reproduce on Mars. One such category is chemolithoautotrophs, which are microorganisms capable of growth through use of inorganic energy sources without input of organic carbon from photosynthesis. Such organisms provide models of the types of microbial life that could potentially thrive in Special Regions on Mars, e.g., in situations where increases in temperature and water activity could make it feasible for utilization of endogenous energy sources on the planet. In simple terms, chemolithoautotrophs extract electrons from inorganic compounds (fuels) and generate metabolic energy through a series of intracellular pathways that conclude with transfer of the electrons to an electron acceptor (oxidant). The energetic feasibility of a given

fuel/oxidant pair (as gauged by  $\Delta G$ , the change in free energy for the overall electron transfer process) is determined by the relative oxidation-reduction potentials ( $p\epsilon^\circ$  values) of the fuel and the oxidant. When  $\Delta G$  is negative, the reaction is energetically feasible; when  $\Delta G$  is zero or positive, the reaction is not feasible. Fig. 5 illustrates this process conceptually, listing several generalized inorganic fuels (e.g.,  $H_2$ ,  $H_2S$ ,  $S^0$ ,  $CH_4$ ,  $Fe^{2+}$ ) and oxidants that are well-known substrates for chemolithoautotrophic metabolism on Earth.

[Figure 5 about here]

In recent years there has been a significant expansion in our knowledge of the range of chemolithoautotrophic pathways and the environments on Earth where they are active. Table 1 provides a brief but comprehensive overview of confirmed or feasible pathways. Virtually all of the reactions depicted have been documented (or are possible) in soil, sediment (freshwater and/or marine), aquifers, or hot spring environments. Of particular significance are recent advances in our knowledge of chemolithoautotrophic iron- and sulfur-oxidizing organisms that utilize nitrate or oxygen for oxidation of insoluble minerals at circumneutral pH (e.g., Weber *et al.*, 2001, Edwards *et al.*, 2003, Shelobolina *et al.*, 2012a; 2012b, Percak-Dennett *et al.*, 2013) i.e., under conditions analogous to those recently identified for the Yellowknife Bay site in Gale Crater (Grotzinger *et al.*, 2013). Also of specific interest to conditions on Mars (cf., Kounaves *et al.*, 2014a) is the ability of hydrogen- and carbon monoxide-oxidizing organisms to

utilize perchlorate ( $\text{ClO}_4^-$ ), an electron acceptor for chemolithoautotrophic growth (e.g., Giblin *et al.*, 2000; Miller and Logan, 2000; Balk *et al.*, 2008). These findings provide examples of Earth organisms that are models of the types of chemolithoautotrophic life that could exist in Special Region situations where oxygen, nitrate or perchlorate may be available to support chemolithoautotrophic life (Jepsen *et al.*, 2007).

[Table 1, here]

Factors such as temperature, pH, and the fuel/oxidant availability dictate which pathways are likely to be active in a given environment. The propensity of evidence suggests that virtually any energetically feasible reaction is likely to be microbially-catalyzed within generally accepted temperature ( $\leq$  ca.  $-20^\circ\text{C}$  to  $120^\circ\text{C}$ ) and pH ( $\leq$  ca. 0 to  $\geq$  ca. 12) limits for life. However, direct demonstration of the feasibility of most of the chemolithoautotrophic pathways listed in Table 1 at extremely low temperatures (i.e., relevant to current conditions on Mars) is limited. Likewise, there has been virtually no work on defining the water activity limits for chemolithoautotrophic metabolisms, all studies having been carried out in systems with water activities close to or equal to one.

[Finding 2-1 Here]

## **2.2 Consideration of microbial “passenger lists”**

The history of Mars exploration dating back to the Viking era has included sampling of thousands of microbial contaminants on Mars-bound spacecraft prior

to their launch. There are several culture collections housing isolates derived from these samples, including: ESA's collection at DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen – German collection of Microorganisms and Cell Cultures) (Moissl-Eichinger *et al.*, 2012), Jet Propulsion Laboratory's (JPL) Phoenix research collection at United States Department of Agriculture – Agriculture Research Service (Venkateswaran *et al.*, 2014), and JPL/Mars Program Office's Mars-related collection archived at JPL, under study in collaboration with University of Idaho (Schubert *et al.*, 2003; Schubert and Benardini, 2013, 2014). Phylogenetic studies of hundreds of bacteria indicate that there is significant and variable diversity of potential microbial passengers on Mars-bound spacecraft, and include a variety of taxa with hardy survival and reproductive capabilities. JPL's DNA-based study of potential passenger lists (including bacteria, archaea, and fungi) is documented in the Genetic Inventory Task Report (Venkateswaran *et al.*, 2012), which utilized and demonstrated state-of-the-art high-throughput molecular methods, but was not intended to be a full census.

[Finding 2-2 Here]

If it were possible to perform a complete census of microbes on spacecraft, then analysis for Special Regions planning could conceivably be narrowed to consider only metabolism(s) and survival strategies of these microorganisms. Current limitations in technology constrain the ability to take a complete census

of microorganisms on and within a spacecraft; until a comprehensive study analyzing both archived DNA as well as contemporary samples with advanced molecular techniques is completed, it is reasonable and prudent to use an inclusive approach by searching all peer reviewed scientific literature for examples of microorganisms on Earth that can function and reproduce at extremely low temperatures (Junge *et al.*, 2004; Methe *et al.*, 2005) or water activity (Kieft, 2002; Potts, 1994). Cataloguing microbial passenger lists utilizing matured molecular methods will serve a purpose for future missions in helping to better identify and evaluate organisms found through robotic spacecraft life detection experiments on Mars, or when samples are returned from Mars.

[Finding 2-3 Here]

### **2.3 Organic compounds on Mars**

Despite annual delivery of  $>2.4 \times 10^8$  g of reduced carbon to the surface of Mars from meteors (Flynn, 1996), only trace organics have been discovered on Mars to date. While earlier studies reported atmospheric levels of CH<sub>4</sub> from  $< 10$  ppbv to a proposed seasonal maximum of 45 ppbv (Formisano *et al.*, 2004; Mumma *et al.*, 2009; Webster *et al.*, 2013), recent measurements via the Tunable Laser Spectrometer on Curiosity have confirmed an upper limit at Gale Crater of only 1.3 ppbv ( $0.18 \pm 0.67$  ppbv; (Webster *et al.*, 2013)). Chloromethane and dichloromethane measured in the Viking pyrolysis experiments after heating surface fine-grained material to 500°C were long attributed to terrestrial

contamination from cleaning solvents (Biemann *et al.*, 1977). However Navarro-Gonzalez *et al.* (2010) suggested that pyrolysis of soils containing perchlorate and organics could account for the Viking results. Coupled with the PHX discovery of perchlorate salts (Hecht *et al.*, 2009), these findings reinvigorated debate about the possible presence of indigenous organics in the martian soils (e.g., Biemann and Bada, 2011; Leshin *et al.*, 2013).

Regardless of the origin of reduced organic carbon compounds, preservation remains a key issue. Whether indigenous, exogenous or terrestrial, extensive chemical oxidation at the surface suggests that remnants of organic carbon would be found only below the surface, either embedded within minerals and hence protected, or as metastable organic salts such as mellitic acid that are more resistant to oxidation but not detectable by gas chromatography-mass spectrometry (Benner *et al.*, 2000; Steele *et al.*, 2012; Ming *et al.*, 2014). In 2012, martian meteorites were shown to contain reduced macromolecular carbon phases (including in one case polycyclic aromatic hydrocarbons) of abiotic/igneous origin based on close association with magmatic mineral grains (Steele *et al.*, 2012). Analysis of fines in aeolian deposits at the Rocknest site by the Sample Analysis at Mars instrument aboard MSL-Curiosity showed concurrent evolution of CO<sub>2</sub> and O<sub>2</sub> that was suggestive of organic material oxidized within the instrument. The origin, however, (martian, interplanetary dust particles or micrometeoritic, or terrestrial contamination) remains unresolved (Leshin *et al.*,

2013; Ming *et al.*, 2014). Carbon isotope results fall intermediate between those of carbonates and reduced carbon signatures from martian meteorites, and may reflect mixing of multiple carbon sources (Leshin *et al.*, 2013). Results from Yellowknife Bay indicate trace levels of chlorinated hydrocarbons, but those detected could be mixtures of reagents added to the samples to transform some compounds to make them easier to analyze (known as “derivatization reagents”), terrestrial contamination from the drill or sample handling chain, or may result from chlorination of martian or exogenous carbon in the Sheepbed mudstone (Ming *et al.*, 2014). The presence of perchlorate salts in martian soils continues to be an important question key to understanding the origin and preservation of organic matter on Mars.

[Finding 2-4 Here]

### **3. Limits to Life on Earth**

#### **3.1 Low temperature limit for terrestrial life (Archaea, Bacteria, Eukarya)**

Mars is a cold place compared to the Earth, so one of the chief challenges for propagation there are the low temperatures, which pose a variety of challenges to cellular systems. As temperature decreases the available thermal energy (enthalpy) of a system decreases, resulting in the increased stability and rigidity of molecules (proteins, DNA, membrane lipids), freezing of water (making it less available), lower rates of diffusion, and decreased chemical reaction rates (for

review see Bakermans, 2012; Cavicchioli, 2006; Russell, 1990). The structural integrity and functionality of cellular systems depend on both the flexibility and stability of their macromolecules, and assemblies thereof. Low temperatures increase rigidity of proteins, lipid bilayers and other macromolecular systems such that metabolic processes can only continue if the optimum flexibility of macromolecular systems is maintained (Fields, 2001; Ferrer *et al.*, 2003; Goodey and Benkovich, 2008; Chin *et al.*, 2010; Struvay and Feller, 2012). In addition, liquid water is the solvent system for enzymes, membranes, *etc.* to function in or for substrates to diffuse through, which is reduced under freezing conditions. Under such conditions pure water crystallizes first, excluding solutes and leaving the remaining water with a higher solute concentration and depressed point of freezing. These waters persist at subzero temperatures in bulk solution or as thin films or veins in soils, sea ice, and glacial ice. While liquid water may exist, ice crystals pose a major physical barrier to the diffusion of molecules (nutrients and wastes) to and from the cell. Chemical reaction rates are particularly impacted by the exponential decrease in thermal energy that accompanies decreasing temperatures, as defined by the Arrhenius equation:

$$k = Ae^{\frac{-E}{k_B T}}$$

where  $k$  is the reaction rate,  $A$  is the pre-exponential term,  $E$  is the activation energy,  $k_B$  is Boltzmann's constant and  $T$  is the absolute temperature in Kelvin.

Despite these challenges, it has long been recognized that terrestrial microorganisms possess adaptations that allow them to function and thrive at low temperatures. To combat the stability and decreased flexibility of proteins and membrane lipids, the molecular structure is altered to increase the disorder within these molecules to maintain fluidity or flexibility and, hence, retain function (Feller, 2007). To contend with reduced water activity and the presence of ice crystals, cells can produce cryoprotectants and anti-freeze proteins (Gilbert *et al.*, 2005; Kuhlmann *et al.*, 2011), and can live in high solute environments (Chin *et al.*, 2010). Furthermore, microorganisms do not appear to be hampered by low rates of metabolic activity which can be sustained for long periods of time ( $10^4$  to  $10^6$  yrs) in various low temperature ecosystems (Johnston and Vestal, 1991).

The actual low temperature limits of terrestrial organisms are currently unknown, primarily due to technological constraints of detecting extremely low rates of metabolism and cell division. But even if the actual low temperature limits of terrestrial organisms are lower than the currently known empirically determined limits, the actual limits may not be relevant to defining Special Regions for the given 500-year time frame because cell division and metabolism would be so slow. For example, cryptoendolithic microbial communities of the Antarctic Dry Valleys (where temperatures rarely exceed  $0^{\circ}\text{C}$ ) successfully invade and colonize sandstones over  $10^3$  to  $10^4$  years (Sun and Friedmann, 1999). Therefore, we examined the currently known empirically determined limits of cell

division and metabolism at low temperatures and did not consider theoretical limits or extrapolations based on current knowledge.

Table 2 provides a list of published, peer-reviewed reports of microbial metabolism at low temperatures that used both direct and indirect measurements of pure cultures and microcosms of environmental samples. Because cell division is difficult to measure directly (via cell counts) at very low temperatures, it is common to examine metabolic processes as indirect measures of microbial activity. However, these indirect measures cannot readily distinguish between cell division, maintenance, or survival metabolism and therefore do not differentiate between low rates corresponding to maintenance and survival or just to long generation times. Studies describing metabolic activity other than cell division were not classified as evidence for cell division, maintenance, or survival metabolism.

[Table 2 about here]

Techniques that measure metabolism requiring the coordinated activity of many enzymes and processes would provide more substantial evidence for active metabolism at low temperatures. Caution must be taken when interpreting data from techniques that measure individual enzymes. Individual enzymes can have temperature optima well outside the growth-temperature range of their parent organism, as in the case of xylanase and aspartate aminotransferase from *Pseudoalteromonas haloplanktis*: while these have temperature optima of 35°C

and 64°C, respectively (Birolo *et al.*, 2000; Collins *et al.*, 2002), the optimum growth temperature of *Pseudoalteromonas haloplanktis* is about 26°C (Piette *et al.*, 2011). This same phenomenon applies at both ends of the growth range; for example, glutamate dehydrogenase from the thermophile *Thermococcus* sp. AN1 can function at temperatures down to -83°C (Daniel *et al.*, 1998) although *Thermococcus* sp. AN1 optimally reproduces at 75°C (Uhl and Daniel, 1999).

[Figure 6 about here]

Temperature limits are not necessarily fixed and multiple factors (such as the physical and chemical parameters of the environment and the physiological condition of cells) will affect what the limits are (Harrison *et al.*, 2013). These include intra- and extracellular solutes that enhance macromolecular flexibility (Chin *et al.*, 2010).

#### 3.1.1. Cell division

To date, cell division has been convincingly demonstrated in the laboratory with pure cultures of isolates by standard measurement techniques such as plate counts or turbidity measurements. One new study since the 2006 report (Mykytczuk *et al.*, 2013) confirms the previously proposed limit for cell division of -15°C. A variety of bacteria (*Firmicutes* and *Gammaproteobacteria*) are capable of cell division at subzero temperatures in solutions with high solute concentrations (Bakermans *et al.*, 2003; Breezee *et al.*, 2004; Mykytczuk *et al.*, 2013). Furthermore, literature not identified in the 2006 report demonstrates cell

division of yeast on frozen surfaces at  $-18^{\circ}\text{C}$  (Collins and Buick, 1989), extending the low temperature limit (Fig 6). Not surprisingly, the doubling times of cells at temperatures of  $-15$  and  $-18^{\circ}\text{C}$  are long (50 and 34 days, respectively) and would likely be longer in an environmental setting. Laboratory experiments on cell division at these low temperatures are difficult due to slow rates, the detection limits of available measurement techniques (plate counts or optical density), and technical challenges associated with working at temperatures below  $0^{\circ}\text{C}$ ; therefore these studies can take a very long time, leading to intrinsic uncertainty in measuring the actual lower limit.

[Finding 3-1 About Here]

### *3.1.2. Metabolic activity*

Microorganisms are known to metabolize at temperatures below the limit for cell division. New studies since the 2006 report extend the previously documented lower temperature limit for metabolic activity from  $-20^{\circ}\text{C}$  to  $-33^{\circ}\text{C}$  (Fig. 6). These studies measured different aspects of metabolism such as DNA synthesis, respiration of acetate, or fluorescence of chlorophyll-a in both pure culture and microcosm studies of organisms from soils, permafrost, and glacial ice from the Arctic and Antarctica (Table 2). One study of note examined genome replication within permafrost microcosms at  $-20^{\circ}\text{C}$  that is highly suggestive of cell division (Tuorto *et al.*, 2014). Another study worthy of notice demonstrated ammonia oxidation activity at  $-32^{\circ}\text{C}$  that was sustained over 300 days, the length of the

experiment (Miteva *et al.*, 2007). The ability of microorganisms to sustain active metabolism at temperatures below  $-33^{\circ}\text{C}$  remains uncertain. While a few studies describing activity of microorganisms at temperatures below  $-33^{\circ}\text{C}$  have been published (Junge *et al.*, 2006; Panikov *et al.*, 2006; Panikov and Sizova, 2007; Amato and Christner, 2009; de Vera *et al.*, 2014) it is not clear if coordinated, sustained metabolism is demonstrated. At the lowest temperatures, rates of metabolism are very low; while some of these levels of activity may support cell division, at present we do not know how to distinguish levels of metabolism that represent very slow cell division from levels that represent maintenance or survival metabolism. Therefore, our finding reflects the empirical low temperature limits of other metabolic activity.

[Finding 3-2 About Here]

### 3.1.3. Chaotropic substances

Numerous types of compounds increase the flexibility of molecules, destabilizing and/or fluidizing them. These compounds, known as chaotropic solutes or chaotropes, can lower the temperature at which organisms are metabolically active (see below). This term ‘chaotrope’ was first used in studies related to the structure of DNA (Hamaguchi and Geiduschek, 1962), and since that time the chaotropic activities of various inorganic and organic compounds ( $\text{MgCl}_2$ , phenol, ethanol, urea, etc.) have been utilized by biochemists for protein solubilisation, denaturation and other *in vitro* protocols (see Harris and Angal,

1989; Sambrook *et al.*, 1989) and as biocides (especially ethanol) and food preservatives (e.g., MgCl<sub>2</sub>, Na benzoate, etc.). All chaotropic substances thus far tested, including MgCl<sub>2</sub>, LiCl, guanidine-HCl, benzyl alcohol, phenol, urea, glycerol and ethanol have been shown to act on macromolecular systems *in vivo* in studies of diverse microorganisms (Hallsworth, 1998; Hallsworth *et al.*, 2003a; 2007; Duda *et al.*, 2004; Williams and Hallsworth, 2009; Bhaganna *et al.*, 2010).

Whereas high concentrations of chaotropic compounds can be benefit microorganisms at low temperatures, at higher temperatures—and at sufficient concentrations—they can be stressful and/or lethal to cellular systems. Studies of the bacterial proteome have demonstrated a specific cellular stress-response intended to counter the stresses induced by the chaotropic activities of chemically diverse substances. This response involves the up-regulation of diverse macromolecule-protection systems (Hallsworth *et al.*, 2003a), a finding that has been confirmed in eukaryotic species (Bhaganna *et al.*, 2010). Furthermore, studies of hydrophobic stressors ( $\log P_{\text{octanol-water}} > 1.9$ ), which partition into the hydrophobic domains of macromolecular systems, demonstrate that they also have chaotropicity-mediated a mode of action; and that a chaotropicity-specific stress response is induced in diverse types of microbial cell to both chaotropic solutes and hydrophobic stressors (Bhaganna *et al.*, 2010; McCammick *et al.*, 2010). Studies of a MgCl<sub>2</sub>-rich, deep-sea hypersaline brine lake (Lake *Discovery*; Mediterranean Sea) reveal that the brine in this location ( $> 5 \text{ M MgCl}_2$ ; water

activity 0.382; temperature 15°C) is highly chaotropic, is devoid of microbial activity and is therefore effectively sterile (Hallsworth *et al.*, 2007). Lake *Discovery* lies 3.58 km beneath the surface of the Mediterranean Sea, and a 1.5-m halocline (0.05 to 5.05 M MgCl<sub>2</sub>) represents the interface between the overlying seawater and the *Discovery* brine (Hallsworth *et al.*, 2007). Studies of the stratified microbial community in the interface between the brine lake and overlying seawater (i.e., the “seawater:Discovery brine interface”) revealed that metabolic activity ceases at 2 to 2.4 M MgCl<sub>2</sub>. Whereas the water activity, osmotic potential, and ionic strength at these MgCl<sub>2</sub> concentrations are biologically permissive for halophilic prokaryotes (e.g., Hallsworth *et al.*, 2007; Daffonchio *et al.*, 2006), MgCl<sub>2</sub> concentrations of > 2.4 M were found to be beyond the chaotropicity window for life (Hallsworth *et al.*, 2007). A recent study of microbiology within the seawater:brine interface at a nearby, but newly discovered, deep-sea hypersaline brine lake (Lake Kryos) reports recovery of mRNA at higher levels of MgCl<sub>2</sub> (i.e., within the range 2.27 to 3.03 M; Yakimov *et al.*, 2014). These concentrations are consistent with studies of the critical concentrations of chaotropic salt, which prevent metabolic activity in the Dead Sea (Oren, 2013). Chaotropic salts and other chaotropic solutes not only stress or prevent activity of microbial systems; at sufficient concentrations they are lethal, and can indeed act as preservation milieu for both macromolecules and whole cells (Duda *et al.*, 2004; Hallsworth *et al.*, 2007). Chaotropicity, therefore, limits

Earth's biosphere in a variety of locations (Hallsworth *et al.*, 2007; Cray *et al.*, 2013a; Lievens *et al.*, 2014; Yakimov *et al.*, 2014) and in this way is comparable with life-limiting parameters such as water activity, pH, temperature, and stressor hydrophobicity. Whereas scales for measurement for most of these parameters were derived some time ago (Celsius, 1742; Berthelot and Jungfleisch, 1872; Sørensen, 1909; Scott, 1957), methodologies and units for the quantitation of chaotropicity and a universal, standard scale for measurement were only recently derived (Hallsworth *et al.*, 2003a; 2007; Cray *et al.*, 2013b).

At temperatures below 10°C, MgCl<sub>2</sub> and other chaotropes have been shown to reduce the temperature minima for cell division by up to 10 or 20°C for diverse microbial species (Sajbidor and Grego, 1992; Thomas *et al.*, 1993; Hallsworth, 1998; Chin *et al.*, 2010) presumably by increasing macromolecular flexibility. This finding is consistent with studies of windows for cell division of a mesophilic bacterium, which were expanded at low temperatures by a comparable margin via the insertion of a chaperonin gene from a psychrophilic species (Ferrer *et al.*, 2003). Chaotropes such as MgCl<sub>2</sub>, CaCl<sub>2</sub>, FeCl<sub>3</sub>, FeCl<sub>2</sub>, FeCl, LiCl, perchlorate, and perchlorate salts (Cray *et al.*, 2013b) are, collectively, abundant in the regolith of Mars. The net chaotropicity of mixed-salt solutions (or, indeed, mixed solutions of other solute types) is influenced by the presence of stabilizing (kosmotropic) solutes which are more polar than water (Oren, 1983; Hallsworth *et al.*, 2003b; 2007; Williams and Hallsworth, 2009; Bhaganna *et al.*, 2010; Bell *et*

*al.*, 2013). It is nevertheless intriguing to speculate whether chaotropic salts on Mars might potentially expand the window for cell division of a microbial psychrophile by reducing the temperature minimum for metabolic activity. This has been demonstrated for Earth microbes at subzero temperatures (Chin *et al.*, 2010) but not yet tested at the known low temperature limit for cell division (-18°C). Cells on Earth, and almost certainly a cell which was located in the relatively dry environments of Mars, can be exposed to saturated concentrations of solutes in brines (including those associated with deliquescent salts), on rock surfaces, within rocks or the subsurface, and in soils for example.

[Finding 3-3 About Here]

### **3.2 Low water activity limit for terrestrial life**

Water is a *sine qua non* for life on Earth and its availability has been accorded central importance vis-à-vis the potential for life on Mars and the definition of Special Regions on Mars. Life's dependence on water is of a diverse nature—for some processes, its fluid properties are important, including transportation of nutrients, waste products, organelles within the organism, and the organism itself, whereas for other processes water is needed as a biochemical consumable, a co-factor, a diluent, a catalyst, or physical stabilizer. A few, but not all, of these needs may be fulfilled by the availability of humidity (water vapor) alone. Water availability is generally quantified as water potential ( $\Psi$ ), which is the free energy of water in a system relative to that of a volume of pure water, expressed in

pressure units (e.g., MPa), or as water activity ( $a_w$ ) expressed as a proportion related to percent relative humidity (RH) as follows:

$$a_w = \text{RH}/100$$

where the relative humidity of an atmosphere is in equilibrium with the water in a system (a solution, a porous medium, etc.). Water potential ranges from 0 (no water) to 1.0 (pure liquid water) and is related to  $a_w$  by a logarithmic function:

$$\Psi = RT(V_w)^{-1} \ln a_w$$

where:

$\Psi$  = water potential (MPa)

$R$  = gas constant ( $8.31 \times 10^{-4} \text{ m}^3 \text{ MPa mol}^{-1} \text{ K}^{-1}$ )

$T$  = temperature (K)

$V_w$  = partial molal volume of water ( $1.8 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1}$ )

Total water potential ( $\Psi_{\text{total}}$ ) is the sum of various components:

$$\Psi_{\text{total}} = \Psi_{\text{solute}} + \Psi_{\text{matric}}$$

where:

$\Psi_{\text{matric}}$  = matric water potential, loss of water availability due to sorption and capillary effects, e.g., desiccation

$\Psi_{\text{solute}}$  = solute or osmotic water potential, the decrease in water availability due to solutes being present in the solution.

As in the 2006 study, water activity continues to be advantageous as a measure of water availability on Mars because it is expressed in units that do not include temperature—although it can be influenced by temperature, as when water is in contact with ice. Water activity can be less than 1.0 due to both solute- and matric effects.

[Table 3 About Here]

An extensive review of the literature, including papers published since 2006, demonstrates that the lowest known  $a_w$  at which terrestrial microbial proliferation has been observed is  $\sim 0.61$  (Table 3, e.g., Stevenson *et al.*, 2014). These findings are divided into microbial responses to solutes, primarily NaCl and sugars, and responses to matric-induced reductions in water.

[Finding 3-4 About Here]

Pitt and Christian's (1968) report of spore germination by the fungus *Xeromyces bisporus* in a sucrose solution at  $a_w = 0.605$  remains the world's record for growth at low  $a_w$ , although spore germination alone may not really amount to cell reproduction (Fig. 7). Linear extension of fungal hyphae at slightly higher  $a_w$  ( $\sim 0.65$ ; Williams and Hallsworth, 2009; Leong *et al.*, 2011) probably better represents the lower  $a_w$  limit for growth. However, as pointed out in previous Special Region reports (Beaty *et al.*, 2006; Kminek *et al.*, 2010), food-related studies conducted in concentrated sugar solutions have little obvious relevance to the growth and reproduction of Earth organisms on Mars, though

some extreme xerophilic fungi such as *Aspergillus penicillioides* inhabit a variety of environments on Earth, most of which are not sugar-rich.

[Figure 7 about here]

Brines are more Mars-relevant, and these have been best studied on Earth for NaCl solutions. Microbial growth is known to occur at all NaCl concentrations, including saturated solutions (~25% w/v, ~ 5 M,  $a_w = 0.75$ ). Halophilic members of the Bacteria, Eukarya, and Archaea are adapted to these extreme salt concentrations, functioning in these brines by excluding  $\text{Na}^+$ , which is inhibitory to many intracellular enzymes, and accumulating intracellular compatible solutes (e.g., KCl, amino acids, glycerol, trehalose, etc.) (Brown, 1976; Harris, 1981; Csonka, 1989). Many other solutes, e.g.,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$  are even more inhibitory than NaCl (as discussed in Section 3.1.3), and thus the lowest documented salt-induced  $a_w$  at which terrestrial microbes can proliferate is 0.75 (Fig. 8).

[Figure 8 about here]

Reductions in  $a_w$  caused by matric effects are more inhibitory to microbial activity and growth than those caused by solute-induced reductions in  $a_w$ , so microbial responses to desiccation offer no challenges to the ~0.605 lower  $a_w$  limit. Desiccation has been well studied in soils, where the inhabitant microorganisms are probably better adapted to matric-induced low  $a_w$  than in any other terrestrial environment. As a soil loses water during desiccation, soil

respiration measured as CO<sub>2</sub> production diminishes to undetectable values at  $a_w = \sim 0.89$  (Griffin, 1981; Manzoni *et al.*, 2012; Moyano *et al.*, 2012, 2013; see Fig. 8).

It must be noted that the measured microbial response here is cellular respiration; as with temperature responses, actual microbial growth likely ceases at a higher water potential. Filamentous fungi, which are able to extend hyphae through air gaps between thin films of water, e.g., in soil litter layers, have been reported to grow at  $a_w$  as low as 0.75 (Harris, 1981; Manzoni *et al.*, 2012).

Causes of inhibition by low matric-induced  $a_w$ —decreases in solute diffusion, cell motility, etc.—are further discussed in relation to thin water films, below.

### **3.3 Other factors affecting life in liquid H<sub>2</sub>O besides $a_w$**

Not all aqueous solutions with activity above the critical value are necessarily supportive of growth and reproduction of microorganisms. In addition to the osmotic stress that may be imposed by a solution with too high or too low concentrations of solutes, there are also considerations specific to the identity of the solutes, themselves. Many solutes that are beneficial or essential nutrients up to some level of concentration may become inhibitory or toxic at higher levels. Adverse effects can arise from a variety of mechanisms, ranging from destabilization of conformation and functional competence of macromolecules (see chaotropic activity, above), to interference with small metabolites. Not all organisms are affected to the same extent. Examples include the halophilic

specialists, which have evolved an extensive repertoire of special capabilities to deal with high solute concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  ions. This does not necessarily pre-adapt them, however, to brines of other simple salts, such as  $\text{MgSO}_4$ ,  $\text{FeCl}_3$ , or  $\text{Ca}(\text{ClO}_4)_2$  which could occur on Mars. Some soluble oxidizers are sufficiently strong to be sterilizing for almost all microbes, ranging from peroxides to hypochlorites (e.g.,  $\text{Ca}(\text{ClO})_2$ ). Transition elements and heavy metals, typically present at only trace concentrations, can facilitate coordination with key ligands as reaction centers for certain enzymatic activities, but become toxic to other functions at higher concentrations.

### **3.4 Atmospheric composition and pressure**

In understanding the prospects for Earth organisms to replicate on Mars, it is important to consider the composition and pressure of the martian atmosphere, which may provide both opportunities and challenges to Earth life. The composition of the martian atmosphere at the surface was originally measured by the Viking landers in 1976 (Owen *et al.*, 1977; Owen 1992), and recent re-measurements by the MSL rover in 2013 were generally consistent with the Viking data and yielded a composition of the five major gases:  $\text{CO}_2$  (96.0%), Ar (1.93%),  $\text{N}_2$  (1.89%),  $\text{O}_2$  (0.145%), and CO (<0.1%) (Mahaffy *et al.*, 2013). In addition, orbital measurements from the CRISM instrument aboard MRO have yielded average values for CO (0.07%) and  $\text{H}_2\text{O}$  (0.03%), but these values are subject to large seasonal variations (Smith *et al.*, 2009b). Several other gases are

found to be present at minor concentrations, such as Ne (2.5 ppm), Kr (0.3 ppm), Xe (0.08 ppm) and ozone (0.03 ppm, but variable) (Owen *et al.*, 1977). Many of the primary gases are likely remnants of the primordial atmosphere (e.g., CO<sub>2</sub>, N<sub>2</sub>, and the noble gases). In addition, through photochemical processes by the action of solar UV radiation on the primary martian gases, some of the minor species have been produced (CO, O, O<sub>2</sub>, O<sub>3</sub>, H, NO) (Krasnopolsky and Feldman 2001).

#### *3.4.1 Methane and hydrogen*

Although most constituents of the martian atmosphere are well known, including their isotopic variability, two gases of astrobiological interest are still not yet well quantified: methane and hydrogen. Both can be important in redox couples for microbial growth. On Earth, methane is produced both by abiotic (e.g., volcanism) and biotic processes (e.g., microbial methanogenesis from CO<sub>2</sub> and H<sub>2</sub>; Ferry, 2010). Thus, methane is potentially an important martian biosignature. Molecular hydrogen (H<sub>2</sub>) is likely produced by UV radiolysis of water vapor in the upper atmosphere, and indeed molecular hydrogen has been detected in the upper martian atmosphere by spectroscopy from Earth-based telescopes (Krasnopolsky and Feldman, 2001). However, molecular H<sub>2</sub> has not to date been measured at the martian surface, although serpentinization of rock in the martian subsurface has been postulated to produce abundant H<sub>2</sub> (Schulte *et al.*, 2006).

#### *3.4.2 Oxygen*

Because molecular oxygen (O<sub>2</sub>) is such a vital need for most multicellular organisms including humans, we tend to forget that on Earth life probably originated and evolved for over one billion years essentially in its absence (Pufahl and Hiatt, 2012). At present, numerous species are known among the bacteria, archaea, and lower eukarya which can grow and reproduce in the absence of O<sub>2</sub> (Fenchel, 2012; Horikoshi *et al.*, 2011). In addition, it is important to note that O<sub>2</sub> is not absent from the martian atmosphere, but is present at a low concentration (ca. 0.00145 volume mixing ratio; Mahaffy *et al.*, 2013). By rough calculation, the pO<sub>2</sub> in the "average" martian atmosphere (which has the pressure of 700Pa, at -10°C) is ~1 Pa, which corresponds to a dissolved O<sub>2</sub> concentration of ~3 nM; in comparison, the O<sub>2</sub> concentration on sea-level Earth (~101.3 kPa, +25°C) is ~250 uM. To put that into perspective, it was recently reported (Stolper *et al.*, 2010) that *Escherichia coli* (bacterial) cells could grow using aerobic respiration at a concentrations of O<sub>2</sub> as low as 3 nM—the same O<sub>2</sub> concentration as on Mars.

[Finding 3-5 About Here]

### 3.4.3 Pressure

In the 2006 MEPAG report, Mars atmospheric pressure was only briefly mentioned as a factor that might affect survival or reproduction of Earth microbes, (Table 1, “Conducive physical conditions”; Beaty *et al.*, 2006). The global “average” pressure on Mars has been variously estimated to be ~ 600-800 Pa, but the actual pressure at a particular location depends on both season and

altitude, generally ranging from ~100 Pa at the top of Olympus Mons to ~1,000 Pa in the Hellas Basin. Lab experiments have shown that most bacteria are unable to proliferate under pressures below ~2,500 Pa using either Earth atmosphere, 100% CO<sub>2</sub>, or simulated Mars atmospheric gas mixtures (Schuerger and Nicholson, 2006; Thomas *et al.*, 2008; Berry *et al.*, 2010; Kral *et al.*, 2011), suggesting the existence of a low-pressure barrier to the growth of Earth bacteria on Mars. Nonetheless, this study reviewed a publication that claimed proliferation of a *Vibrio* sp. under the low pressure of 1-10 Pa (Pavlov *et al.*, 2010), and two reports were published in 2013 describing proliferative cell division under a low-pressure simulated Mars atmosphere (700 Pa, 0°C, and anoxic CO<sub>2</sub>) by six *Carnobacterium* spp. isolates from Siberian permafrost (Nicholson *et al.*, 2013) and by a laboratory strain of *Serratia liquefaciens* (Schuerger *et al.*, 2013). These results suggest that the low-pressure barrier is not in any way absolute, and that variations in atmospheric pressure cannot (at present) be used to define Special Regions in one part of Mars versus another.

[Finding 3-6 About Here]

### **3.5 UV radiation on the surface of Mars**

During the day, Mars is bathed in strong ultraviolet (UV) light. The wavelength of UV radiation on Mars extends from ~190-400 nm, encompassing UV-C, -B, and -A wavelengths. Given that the martian atmosphere is thin, CO<sub>2</sub>-rich, and ozone-poor, the UV reaching the surface of Mars has a ~1000-fold

greater biocidal effect than on Earth (see Beaty *et al.*, 2006). Before 2013, data from direct measurements of UV spectrum and intensity at the surface had not been available, so ground-based simulations were based on various models (Kuhn and Atreya, 1979; Appelbaum and Flood, 1990; Cockell *et al.*, 2000; Patel *et al.*, 2002) which were generally in good agreement with each other. Subsequent direct measurements of UV, which were made by MSL's Rover Environmental Monitoring Station (REMS), were found to differ from the models by less than a factor of 2 (María-Paz Zorzano, personal communication).

Experiments conducted prior to 2006 had shown that hardy spores of organisms that were actual spacecraft contaminants could be deposited on spacecraft surfaces and exposed to UV closely replicating the spectrum and intensity of Mars. The results of these experiments demonstrated that: (1) unshielded spores were rapidly inactivated within a few minutes to a few hours, and; (2) relatively thin layers (on the order of less than a millimeter) of UV-opaque materials such as dust or regolith could effectively shield microbes from UV (see below and references given in Table 4).

[Table 4 About Here]

Numerous studies published since the 2006 MEPAG report have measured the survival of various microorganisms subjected to simulated Mars UV exposure (e.g., Diaz and Schulze-Makuch, 2006; Tauscher *et al.*, 2006; Moores *et al.*, 2007; Pogoda de la Vega *et al.*, 2007; Fendrihan *et al.*, 2009; Gomez *et al.*, 2010;

Johnson et al., 2011; Kerney and Schuerger, 2011; Osman et al., 2008; Peeters et al., 2010; Smith et al., 2009a), but none of those studies have led to changes in findings (1) and (2) above. Since UV light may be received everywhere on the surface of Mars, it is not a good discriminator regarding the presence or absence of Special Regions on Mars, although its effects on the martian surface chemistry may be profound all over Mars.

[Finding 3-7 About Here]

### **3.6 Ionizing radiation at the surface**

In the 2006 MEPAG report, it was stated that the surface of Mars is “significantly influenced by galactic cosmic radiation at all times,” and that “for organisms near or at the surface, long-term exposure to galactic cosmic rays (GCR) and solar particle events (SPEs) will certainly increase lethality and reduce viability” (Beatty *et al.*, 2006). In 2012-13, direct measurements of the flux of ionizing radiation on the surface of Mars were made using the RAD instrument carried on the MSL mission (Hassler *et al.*, 2014). During a 300-sol period, the RAD instrument detected a relatively constant ionizing radiation flux of ~0.18-0.225 mGy per day, composed almost exclusively of GCR; a single SPE on Sol 242 was recorded as a transient spike to 0.26 mGy per day. Evaluation of long-term integrated solar energetic particle (SEP) doses for asteroids show that they do not exceed the GCR dose except near the surface (Clark *et al.*, 1999), and that the martian atmosphere provides sufficient shielding that the total SEP dose is less

than double the GCR dose. Over a 500-year time frame, the Mars surface could be estimated to receive a cumulative ionizing radiation dose of less than 50 Gy, much lower than the LD<sub>90</sub> (lethal dose where 90% of subjects would die) for even a radiation-sensitive bacterium such as *E. coli* (LD<sub>90</sub> of ~200-400 Gy) (Atlan, 1973). Accordingly, it can be stated that the RAD data showed that the total surface flux of ionizing radiation is so low as to exert only a negligible impact on microbial viability during a 500-year time frame (Hassler *et al.*, 2014). These findings were in very good agreement with modeling studies (Dartnell *et al.*, 2007; Norman *et al.*, 2014).

[Finding 3-8 About Here]

### **3.7 Polyextremophiles: Combined effects of environmental stressors**

In the majority of Mars simulations studies, parameters (pressure, temperature, UV, etc.) have been applied either singly or in at most a combination of two or three. Thus, at present it is unknown how microorganisms respond to the complete suite of Mars environmental conditions applied simultaneously. For example, there are no direct measurements of the highly active species predicted by photochemical models of the interaction of solar UV with atmospheric constituents to produce free radicals, atomic species, ions, and even molecular oxidants (such as O<sub>3</sub>). Some of these species may be catalytically or reactively destroyed by interaction with soil grains, but this is largely unknown at this time, and may have primary or secondary interactions with frost on the martian surface.

It may be that these species have destroyed organic material in the upper mm to meters of martian soil, and over time may be able to sterilize that layer, as well.

[Table 5 About Here]

The term polyextremophile refers to microorganisms that possess some type of resistance to or repair mechanism for more than one challenging environmental circumstance (Harrison *et al.*, 2013), some of which are listed in Table 5. These also may include hypertolerant organisms, which can withstand extremely concentrations of a substance considered to be toxic to life, such as arsenic (Drewniak *et al.*, 2008). In some cases, microorganisms may possess what appears to be a single main mechanism that confers resistance to more than one condition, e.g., salt tolerance and radiation resistance (Rainey *et al.*, 2005). In other cases, microorganisms seem to have developed separate mechanisms to address different conditions but are experiencing them simultaneously in their environments. Examples include *Psychrobacter* LOS3S-03b isolated from deep sea hydrothermal vents that has been studied for resistance to heat shock, desiccation, H<sub>2</sub>O<sub>2</sub>, and UV and ionizing radiation (La Duc *et al.*, 2007). Yuan *et al.* (2012) have noted that the organism *Deinococcus gobiensis* has resistance to both gamma and UV radiation and that this resistance appears to be related to the same mechanisms. Jones *et al.* (2011) developed the idea of temperature and pressure phase space in an attempt to assess the interactions of resistance to both of these environmental conditions. A study that attempted to compare resistance

to temperature, pH, salt (NaCl) concentrations, and pressure (Harrison *et al.*, 2013) had concluded that their study “...reveals a fundamental lack of information on the tolerance of microorganisms to multiple extremes that impedes several areas of science...” Understanding how microorganisms respond to multiple extremes is an important consideration for planetary protection. Any organisms on spacecraft would experience exposure to multiple extremes (radiation, desiccation, etc.) and their ability to tolerate and/or repair damage could affect their ability to survive transit to a Special Region (natural or spacecraft induced). A broader understanding of polyextremophiles could redefine our limits to life and in turn Special Regions on Mars.

[Finding 3-9 About Here]

### **3.8 The issue of scale: Detecting microbial microenvironments**

Martian environments we can detect from orbit are at what might be called “landscape-scale”. The quantification of these environments depends on the nature of the instrument package used to detect them, but the detectable scale is typically one of meters to kilometers. Detected environmental conditions can also be scale-dependent over time because of kinetic factors—where the environment is not yet (and may never be) in thermodynamic equilibrium, and in any event is characterized by temperatures and pressures unfamiliar to Earth organisms (including humans).

[Table 6 About Here]

In contrast, organisms that may be carried by spacecraft can be driven by processes undetected from space, or governed by environmental extremes not previously encountered. For example, the environmental conditions of relevance to a microbe are measured at a scale of  $10^0$ - $10^2$  microns, which cannot be directly observed from orbit (see Table 6). Likewise, orbital observations, and even landed missions working for only a short time (e.g., 150 days to 10 years) may never detect processes taking place on the timescale of decades or centuries, or may have a revisit-time between observations of a particular surface location that is months or years long. In each of these cases, critical details will be missed because of the mismatch of scales between what is measured and the technology used to measure it.

[Finding 3-10 About Here]

### *3.8.1 Possible microscale environments on Mars*

Despite the inherent difficulties of exploring an entire world scientifically, Mars is gradually giving up clues to the possibility of environments that may be capable of supporting Earth organisms. At present, Mars exploration is focused more on questions regarding ancient habitability than on questions of present-day environments, so certain data may be lacking to assess them completely, but it is clear that there are candidates that must be examined. Accordingly, a set of seven microenvironments that either do or might exist naturally on Mars was defined for characterization and evaluation as part of this study (Table 7). The following

sections of this report evaluate the possibility that these microenvironments exist on Mars, and if so, whether their natural environmental conditions are within bounds that allow for the reproduction of terrestrial microbes. An additional set of four microenvironments that might be created by different kinds of exploration activities is also included in Table 7.

[Table 7 About Here]

### *3.8.2 Vapor-phase water and its use by Earth organisms*

Desert environments on Earth are demanding habitats for life due to their limited water availability. Under dominating aridity, liquid water is observed either during periodic rainfall events, under foggy conditions, or as condensation on surfaces by dew formation. Atmospheric relative humidity (water vapor) can increase at night due to atmospheric cooling but is normally low. The resulting water stress results in the restricted diversity of desert life, dominated by soil- and rock-surface microbial communities that are defined by their physical location with regard to those surfaces, and include biological soil crusts, hypoliths, epiliths, endoliths, and bio-aerosols (Pointing and Belnap, 2012). Table 8 cites some of the available literature regarding microbial metabolism and growth in deserts on Earth.

[Table 8 about here]

While these conditions exclude many life forms, the poikilohydric nature of lichens allows them to live in such extremely arid climates without suffering the

damage that can be caused by periods of dryness punctuated by episodic exposure to elevated moisture conditions. These are best characterized in areas where dew condensation or fog occasionally occur (usually at night), and the presence of liquid water allows for hydration and dark respiration followed by CO<sub>2</sub> fixation associated with net photosynthesis in the early part of the day. This activity subsequently ceases as temperatures rise and humidity levels drop, leading to desiccation due to water loss through evaporation (Lange *et al.*, 2006; Lange *et al.*, 1990).

Under more extreme conditions where moisture is scarce it has been shown that lichens are metabolically active in the absence of liquid water, down to 70% relative humidity (Lange 1969, Lange *et al.*, 1970, Nash *et al.*, 1990, Palmer and Friedmann 1990, Lange *et al.*, 1994, Lange and Redon 1983, Redon and Lange 1983). Lichens are symbioses between fungi and algae or cyanobacteria (referred to as phycobionts). Lichens specifically with algal phycobionts appear to function at these lower relative humidities, whereas those with cyanobacterial phycobionts have a higher threshold near 90% (Hess, 1962; Palmer and Friedmann, 1990). While all can revert to activity through contact with liquid water, it has been shown that uptake of water vapor alone can reactivate photosynthesis in lichens with an algal phycobiont (Butin, 1954; Lange and Bertsch, 1965; Lange and Kilian, 1985; Nash III *et al.*, 1990; Schroeter, 1994), whereas lichens with cyanobacterial phycobionts do not exhibit the same

universal capacity and appear to require liquid water to activate photosynthesis (Lange *et al.*, 1993; Lange *et al.*, 2001; Lange and Kilian, 1985; Lange *et al.*, 1986; Lange *et al.*, 1990; Lange and Ziegler, 1986; Schroeter, 1994).

Microscopic examination of both types of lichens has shown this to be due to the inability of cyanobacteria to attain turgidity when hydrated with water vapor alone (Büdel and Lange, 1991). However, it has also been shown that a cyanobacterial phycobiont isolated in the laboratory can achieve turgor and photosynthesize under conditions of high humidity (Lange *et al.*, 1994). Such work brings validity to earlier studies showing that cyanobacteria can photosynthesize under arid conditions, including biological soil crusts and cryptoendolithic habitats (Brock, 1975; Palmer and Friedmann, 1990; Potts and Friedmann, 1981).

[Finding 3-11 About Here]

While photosynthetic activity in the absence of liquid water has been documented in arid climates of temperate regions where local humidity can be high (Lange and Redon, 1983; Redon and Lange, 1983), metabolic activity can also occur at subzero temperatures where water exists in a solid phase as snow or ice, often under snowcover (Kappen, 1989; Kappen *et al.*, 1986; Kappen and Breuer, 1991; Kappen *et al.*, 1990; Kappen, 1993; Pannowitz *et al.*, 2003; Schroeter and Scheidegger, 1995). While melting of snow and ice can lead to moistening (Lange, 2003), water vapor by itself supports metabolic activity under

cold temperatures (Kappen *et al.*, 1995) whereby a vapor gradient forms between ice and the dry lichen thallus (Kappen and Schroeter, 1997).

The ability to attain net photosynthesis using water vapor alone as well as survive long periods of desiccation are important survival strategies for lichens in desert habitats. Lichens with algal phycobionts appear to attain positive net photosynthesis under lower relative humidity conditions than those with a cyanobacterial phycobiont, and experience much higher rates of photosynthesis when exposed to higher humidity levels. This suggests that they are the best opportunists to survive under the most arid conditions on the planet. While the limits for activity have been well defined, evidence for cellular reproduction (i.e., propagation) in the complete absence of liquid water remains to be confirmed—but may be possible, and could have significant implications with respect to the existence of Special Regions on Mars.

[Finding 3-12 About Here]

### *3.8.3 Ice-related microenvironments*

Ice can contain unfrozen water in a vein network between ice crystals where solutes concentrate that may be a possible habitat for microorganisms (Mader *et al.*, 2006; Price, 2000). Various studies conducted since 2001 support the idea that microorganisms can be active within ice. For example, bacteria have been found to exist and metabolize within briny veins and inclusions in sea ice (Junge *et al.*, 2004; Junge *et al.*, 2006). The presence of anomalous gas concentrations in

glacial ice also suggest that microorganisms can metabolize within ice (Sowers, 2001; Campen *et al.*, 2003; Tung *et al.*, 2005 Tung *et al.*, 2006 Miteva *et al.*, 2007; Rohde *et al.*, 2008). However, it is unlikely that life can reproduce within crystalline ice without the presence of liquid water. All ice-related microenvironments are constrained by the low temperature limit defined in Section 3.1 and the water activity limits defined in Section 3.2.

#### *3.8.4 Brine-related microenvironments*

Brine-related microenvironments can occur at a variety of scales, from large volumes of brine down to fluid inclusions in salt or ice crystals (e.g., Hallsworth *et al.*, 2007; Gramain *et al.*, 2011; Lowenstein *et al.*, 2011; Yakimov *et al.*, 2014). Determining whether a terrestrial microbe could reproduce in such an environment is almost entirely dependent on the physical chemistry and thermodynamics of the brine, however, rather than the physical scale of the brine pocket. As such, constraints on this microenvironment are described in Section 3.8.1 of this report.

#### *3.8.5 Thin films*

Observations and models indicating small amounts of transient water on martian surfaces, including spacecraft surfaces (e.g., the PHX lander), raise the question of whether these droplets and thin films of water could support proliferation of terrestrial microbes. The answer lies in our understanding of microbial responses to low water activity, as discussed in Section 3.2. Loss of

water in a system dominated by one or more solid surfaces, i.e., decrease in matric water potential or water activity, exemplified by desiccation of a porous medium (soil, martian regolith, food, etc.), is more inhibitory to microbial activity than equivalent decreases in  $a_w$  caused by solutes such as NaCl or sugars (Harris *et al.*, 1981). As a porous medium loses water during desiccation, the thickness of water films diminishes. Water film thicknesses vary primarily as a function of water potential (or water activity), but are also influenced by surface roughness, surface hydrophobicity, temperature, texture, and other factors; water film thickness are also not uniform, so an average water film thickness is measured or calculated (Papendick and Campbell, 1981; Harris, 1981; Tokunaga, 2012). Considering a range of data for average water film thicknesses (Tokunaga, 2012) and converting water potentials to water activities, it is clear that average water film thickness declines sharply as  $a_w$  declines from 1.0 to 0.90, and that the highest value of water film thickness estimate at  $a_w = 0.9$  is ~15 nm (Fig 9).

[Fig. 9 here somewhere]

This is one-tenth or less of the diameter of the smallest terrestrial microbial cells (Kieft, 2000). Solute diffusion and cell motility within such thin films are nearly zero (Griffin, 1981), and thus microbes are trapped without access to external nutrients. Moreover, they are likely losing water to the thin films rather than gaining the requisite water for population growth (increase in abundance and biomass). Empirical data supporting this view include the repeated finding that

soil respiration (CO<sub>2</sub> production by inhabitant microbes) declines to unmeasurable values as soils are desiccated to  $a_w = 0.89$  and lower (Sommers *et al.*, 1981; Manzoni *et al.*, 2012; Moyanao *et al.*, 2013). Any solutes within the water of the thin films would further decrease the  $a_w$  and further inhibit microbial activity. The overall conclusion regarding water films is that water activity remains the relevant fundamental parameter influencing water film thickness and microbial responses.

[Finding 3-13 about here]

### 3.8.6 Groundwater

Approximately 50% of the Earth's total biomass exists as subsurface prokaryotic life, much of which is found within unconsolidated sediments and groundwater (Whitman *et al.*, 1998). Rock-water interactions in the subsurface provide numerous substrates (e.g., H<sub>2</sub>) to support chemosynthetic microbial activity that may include denitrification, manganese reduction, iron reduction, sulfate reduction, and methanogenesis (Stevens and McKinley, 1995; Nealson *et al.*, 2005; Lin *et al.*, 2006; Chivian *et al.*, 2008). Methane can be generated from H<sub>2</sub> and inorganic carbon via Fischer-Tropsch-type synthesis, and this too can fuel subsurface activities (Sherwood Lollar *et al.*, 2002). It is thought that groundwater was abundant on Mars during the Noachian and Hesperian periods (Carr and Head, 2010), and likely persists at some depth and quantity today (Clifford *et al.*, 2010, Lasue *et al.*, 2013). Based on potential scenarios for

groundwater to exist (Michalski *et al.*, 2013) and assuming that the necessary minerals, nutrients, and energy are present (Fisk and Giovannoni, 1999), these groundwater systems may support similar microbial metabolisms (Boston *et al.*, 1992) including hydrogen-based methanogenesis (Chapelle *et al.*, 2002) or anaerobic methane oxidation (Marlo *et al.*, 2014).

Evidence for groundwater activity on Mars include surficial expressions such as recurring slope lineae (McEwen *et al.*, 2011) as well as mineral deposits including sulfates, clays, and carbonates. Assuming that these minerals formed as a result of chemical supersaturation and subsequent precipitation from the aqueous phase, such deposits provide important evidence for past or present groundwater activity. Similar deposits associated with groundwater spring activity and subsurface microbial communities are found on Earth (Chivian *et al.*, 2008; Farmer, 2013; Janssen and Tas, 2014). While it is hypothesized that groundwater on Mars would be briny (Burt and Knauth, 2003), brines can support subsurface microbial life on Earth (Brown, 1976; Csonka, 1989; Bottomley *et al.*, 2002; Katz and Starinsky, 2003; Lin *et al.*, 2006; Onstott *et al.*, 2003; Li *et al.*, 2012). In the absence of conclusive evidence for groundwater activity, comparison of surficial deposits associated with groundwater activity to those observed on Mars make them important targets for study by potentially linking them to deep subsurface groundwater supporting microbial life on Mars.

[Figure 10 About Here]

### *3.8.7 Condensation and melting*

Whereas the martian environment is dry and cold, and the atmospheric pressure of Mars is quite low by Earth standards, Mars is not always so cold as to freeze water, and outside of the Tharsis bulge and Olympus Mons (and especially in Hellas and other basins) the atmospheric pressure is generally high enough to allow any unfrozen water to exist as a liquid for short periods of time before it either evaporates or boils away. This is a dynamic process, and the persistence of water would be influenced by the existence of solutes in the water or the presence of nearby ice, while its evaporation or boiling would be expected to be affected by insolation or other sources of heat. Fig. 10 shows the narrow window above 608 Pa (0.006 atm) where liquid water can be stable when temperatures are above 0°C and below about 7°C.

With such a narrow window for its stability, it would seem that water would have to be delivered through the atmosphere to a specific location for liquid water to be found at that spot, and at this point in time there are no expectations that liquid water as rain will fall as part of the water cycle on Mars. Snow, however, has been detected on Mars (see Section 4.11). If snow melting yields liquid water on the surface of Mars, even periodically for only a short time, that water could be available for microbial use and define (for however short a time) a Special Region on Mars.

[Finding 3-14 Here]

### 3.8.8 *Water in minerals*

Minerals can be sources of water and so the question arises as to how biologically available this mineral-associated water is. Swelling clay minerals like smectite serve as a good test case because they can hold more water than nearly any other minerals due to their extremely high surface-to-volume ratio.

[Figure 11 about here]

In Figure 11, the strong silicate (red and yellow) tetrahedra shown are connected by octahedrally coordinated aluminum and magnesium to form strong continuous sheets. The sheets are held together by water molecules and cations in the interlayer. Water molecules are also adsorbed on the surface of the tetrahedral sheets that may or may not form a thin film of water molecules depending on the availability of water in the environment. The bioavailability of this surface-sorbed water is related to other thin film water, as discussed in Section 3.8.5. This outer surface-associated water is only of use to microbes at water activities above  $\sim 0.9$ . The hydrogen found between the tetrahedral layers occurs as  $\text{OH}^-$  and is not released until the clay is destroyed at high temperatures. The water molecules between the layers can be released over time, under low humidity/low  $a_w$  conditions and the distance between the tetrahedral sheets decreases correspondingly. This contraction of the clay often causes mud cracks to appear in the sediment. The interlayer water is not directly available to microbes due to tight binding to the clay minerals and the thinness of the interlayer ( $\leq 4$  nm).

Structural water within the mineral is not biologically available. Minerals such as pyroxene can also have surface-sorbed water but less of it than clay minerals due to the lower-surface-to-volume ratio. The conclusion that we can draw here is that mineral-associated water held by clay minerals does not form an exception to the previous conclusions about the effects of  $a_w$  or of water in thin water films, and therefore is not biologically available outside of those constraints.

### **3.9 Asynchronous access to resources by organisms and its potential significance to Special Regions**

Physical, chemical, and biological processes occur in response to relatively rapid changes in environmental conditions, such as diurnal variation in temperature and relative humidity. The processes are frequently not instantaneous, and the details of the kinetics may have biological consequences. Lag times between these processes (given non-equilibrium conditions) could provide intervals of favorable conditions for biological function. However, the extent to which organisms can retain a particular favorable condition or resource while waiting for a favorable state of another condition to occur is poorly studied. The primary asynchrony of significance for purposes of assessing Special Regions on Mars revolves around the acquisition of water from an extremely dry or cold environment followed by a subsequent overlap and maintenance of that liquid acquisition with episodes of temperatures high enough for cellular reproduction. In other words, can organisms “*wait it out*” between periods of higher relative

humidity at sub-growth temperature, and a later rise in temperature that permits growth but where the relative humidity is below usable water activities?

[Figure 12 about here]

### *3.9.1 Abiotic water-trapping mechanisms*

Some surface properties can facilitate condensation of vapor phase materials even when the conditions of the bulk material do not favor such condensation (e.g., Park et al., 2007; Humplik et al., 2011). Hallmark characteristics of such a situation include physical or chemical properties (e.g., surface roughness, or three-dimensional structures) that can operate in two different ways: 1) cause enhanced attraction of vapor-phase or liquid phase water to surfaces or materials or 2) retard the evaporation or sublimation of water back to the environment. Passive microniche water-trapping capacities include several examples. First, porous rock (e.g., sandstone) has been shown to absorb occasional frost or snow (cf., Friedmann *et al.*, 1987; see Fig. 12). The complex three-dimensional fine structure of the rock physically retards evaporation because of extensive intergranular spatial conduits and high surface area. A second example is found in desert or rock varnishes, which are surface coatings that form on arid land rock surfaces and are composed of metal oxides (particularly iron and manganese) with often a silica glaze over the metal oxide layers (Dorn, 1991, 2007a; Liu and Broecker, 2000). On Earth, such varnishes are facilitated by the presence of microbial communities driven by photosynthesis and comprised of a number of

different types of organisms (Spilde *et al.*, 2013; Northup *et al.*, 2010; Liu *et al.*, 2000; Garvie *et al.*, 2008; Dorn and Krinsley, 2011; Kuhlman *et al.*, 2008). The silica glazes that sometimes overlie the oxide, clay, and microorganisms (Perry *et al.*, 2006; but see Dorn, 2007b) are patchy at the micron and tens of micron scales, thus allowing penetration of water but also acting to inhibit evaporation or sublimation.

[Finding 3-15 here]

### *3.9.2 Biotic water-trapping mechanisms*

In addition to abiotic processes, biotic physico-chemical water-trapping capacities exist based on some type of highly absorptive biomolecules that trap fluid in one of two ways: 1) biomolecules with an intrinsically high affinity for water and three dimensional structures that helps to retain the water, or 2) layered biologically produced structure composed of impervious or less permeable materials. Many types of glycoproteins hold water (well described in Antarctic fishes) because of their chemical affinity for it, and some can act as antifreeze compounds (e.g., Devries, 1971; Davies and Sykes, 1997). Mucins and compounds with sugar groups hold water and retard enzymatic digestion (Derrien *et al.*, 2010), both properties enhancing water retention within a microorganism. Some organisms can also produce proteins which bind to ice crystals (known as ice binding proteins) inhibiting recrystallization (i.e., Jia, *et al.*, 1996), which enables cells to maintain a liquid environment at lower temperatures and has been

shown to retain brine within sea ice (Janech *et al.*, 2006; Raymond *et al.*, 2008). Ice-nucleation proteins in some plant-pathogenic bacteria (Lindow *et al.*, 1982; Gurian-Sherman and Lindow, 1995) and in some lichen fungi (Kieft, 1988) serve as templates for the ordering of water into crystal lattices at relatively warm temperatures ( $\sim 3^{\circ}\text{C}$ ), and in the case of the lichens, these may enhance moisture acquisition. Macroscopic structures like microbial mats, cyanobacterial sheaths and trichomes and thick lichen thalli can allow penetration of fluid but can act as a low permeability barrier to re-evaporation or sublimation (Ortega-Calvo *et al.*, 1991; Verrecchia *et al.*, 1995; Stoltz, 2000).

#### **4. Observed Martian Phenomena Potentially Related to Naturally Occurring Special Regions**

[Figure 13 about here]

##### **4.1 Recurring slope lineae**

RSL are narrow (<5 m wide), dark markings on steep ( $25^{\circ}$ - $40^{\circ}$ ) slopes (Fig. 13) that appear and incrementally grow during warm seasons over low-albedo surfaces, fade when inactive, and recur over multiple Mars years (McEwen *et al.*, 2011). They are considered “confirmed” when many (>10) features are seen to grow incrementally on a slope, fade, and recur in multiple years. RSL are called “partially confirmed” when either incremental growth or recurrence has been observed thus far, but not both. There are many processes that form relatively

dark lines on steep slopes, including slow and rapid dry mass wasting. Therefore, observing the peculiar temporal behavior is essential for definite RSL identification. They often follow small gullies, but no topographic changes in these gullies have yet been detected via 30 cm/pixel images from MRO's HiRISE. There are some features that are RSL-like yet do not fit all criteria; for example, in Aram Chaos, slope lineae only grow a bit at their tips and have not faded for over 2 Mars years.

[Figure 14 about here]

There are 3 geographic groups of confirmed RSL. Those in the first group appear and lengthen in the late southern spring through summer from 48°S to 32°S latitude, favoring equator-facing slopes—times and places with peak diurnal surface temperatures ranging from >250 K to >300 K. Over 2012-2013, active RSL have been confirmed in equatorial (0°-15°S) regions of Mars, especially in the deep canyons of Valles Marineris (McEwen *et al.*, 2014a). The equatorial RSL are especially active on north-facing slopes in northern summer and spring and on south-facing slopes in southern spring and summer, following the most near-to-direct solar incidence angles on these steep slopes. Some of these lineae are especially long, over 1 km, following pristine gullies. More recently RSL have been confirmed near 35°N in low-albedo Acidalia Planitia, on steep equator-facing slopes; these RSL are active in northern summer (McEwen *et al.*, 2014b).

The global distribution of RSL (Fig. 14) shows them below 2.6 km altitude, and only on low-albedo (low-dust) surfaces.

The fans on which many RSL terminate have distinctive color and spectral properties in MRO/CRISM data, but lack distinctive water absorption bands (Ojha *et al.*, 2013). Ferric and ferrous spectral absorptions increase with RSL activity, perhaps due to removal of a fine-grained surface component during RSL flow, precipitation of ferric oxides, and/or wetting of the substrate.

All confirmed RSL locations have warm peak daily temperatures (typically  $>273$  K at the surface) in the seasons when RSL are active. However, most times and places with these properties lack apparent RSL (Ojha *et al.*, 2014), so there are additional, unseen requirements for RSL formation. We do not know what time of day RSL are actively flowing, so the temperature of any water associated with them is not known. There is no observational constraint on salt concentration. The peak RSL activity in the mid-latitudes corresponds to the season of peak temperatures in the shallow subsurface ( $<1$  m) rather than at the surface, consistent with melting ice or heating hydrated salts in the shallow subsurface.

Laboratory experiments show that very small amounts of water or brines darken basaltic soils but may only produce weak water absorption bands undetectable in ratio spectra after partial dehydration during the low-humidity middle afternoon conditions when MRO observes (Pommerol *et al.*, 2013b;

Massé *et al.*, 2014a). No entirely dry process is known to create such slowly or incrementally advancing seasonal flows or their rapid fading, but the RSL bear some similarities to avalanches on martian dunes (Chojnacki *et al.*, 2014) and to slope streaks on dust-mantled slopes (Mushkin *et al.*, 2010). Lab experiments show that boiling brines may trigger dry flows under martian atmospheric pressure (Massé *et al.*, 2014b), suggesting a mechanism for RSL formation with minimal water.

The primary questions about RSL for Special Region consideration are whether they are really due to water at or near the surface, and if so, what is the temperature and water activity. All observations can be explained by seeping water, and no entirely dry model has been offered, but there is no direct detection of water. If they are due to water, a key problem is where the water comes from and how is it replenished each year.

Below are a few hypotheses:

#### *4.1.1 Deliquescence*

This phenomenon has been reported as the source of some water tracks in the dry valleys of Antarctica, which appear very similar to RSL (Levy, 2012). This hypothesis is attractive as it could explain some RSL that begin near the tops of ridges or hills. The seasonal variation in the atmospheric column abundance of water vapor does not match the RSL activity (Toigo *et al.*, 2013; McEwen *et al.*, 2014a) and the quantities of water vapor are extremely small (~1% of that over

Antarctica). However, deliquescence might re-hydrate shallow subsurface chloride hydrates that liquefy upon seasonal heating (Wang *et al.*, 2014), and RSL might be triggered by small amounts of water (Massé *et al.*, 2014b). In this scenario the water activity would be quite low, not habitable to known terrestrial organisms.

#### *4.1.2 Melting frozen brines from a past climate*

This model (Chevrier and Rivera-Valentin, 2012) explains the observation that peak RSL activity corresponds to seasons of peak temperatures in the shallow subsurface. However, it is difficult to explain how such ices could remain present for  $>10^5$  years on such warm slopes, particularly if they annually melt extensively enough to produce long flows. The water activity would again be low.

#### *4.1.3 Fault-controlled migration of deep (ancient?) brines*

Brines are expected to exist in the martian crust (Burt and Knauth, 2003), and could migrate to the surface along certain pathways and reach the surface on steep slopes. In a few mapped sites,  $>80\%$  of the RSL are within 50 m of an observed fault (Watkins *et al.*, 2014). In this scenario, water activity could be high enough for terrestrial organisms.

#### *4.1.4 Brine convection*

This process occurs in Earth's ocean and should occur in the subsurface of Mars if it is saturated with brines, depositing pure (not salty) ice near the surface (Travis *et al.*, 2013). Saturated ground is highly unlikely in most regions where

RSL are located, although fault-controlled movement of brines might also replenish shallow ice. Melting pure ice would produce low-salt water with a high water activity, potentially habitable.

#### *4.1.5 Ice replenished by vapor transport*

This model (Grimm *et al.*, 2014; Stillman *et al.*, 2014) also forms pure ice near the surface, but vapor transport is too slow (Hudson *et al.*, 2009) to explain yearly recurrence of the quantities of water envisioned by these authors.

[Finding 4-1 Here]

[Figure 15 About Here]

## **4.2 Gullies**

A class of geologically youthful martian landforms consisting of erosional alcoves, straight or sinuous channels, and depositional aprons (Fig. 15) was first described by Malin and Edgett (2000). These were compared to terrestrial gullies formed through the action of liquid water, and a range of potential martian water sources was proposed, including shallow groundwater aquifers (Malin and Edgett, 2000) or the melting of ground ice (Costard *et al.*, 2002) or snowpack (Christensen, 2003). Most recent studies arguing for a wet gully origin favor snowmelt as the water source (e.g., Dickson and Head, 2009). However, dry models have also been proposed, whether related to CO<sub>2</sub> volatilization (Hoffman, 2002; Cedillo-Flores *et al.*, 2011; Diniega *et al.*, 2013) or mass wasting of either

frost-coated (Hugenholtz, 2008) or volatile-poor granular material (e.g., Treiman, 2003; Pelletier *et al.*, 2008).

[Figure 16 About Here]

Gullies are widespread and occur at all latitudes, but are most abundant in the middle latitudes (Fig. 16). They are found in ~100 times as many locations as RSL (McEwen *et al.*, 2011). While these two types of features often co-occur in southern mid-latitude impact craters, most commonly the alcove-channel-apron gullies dominate the pole-facing slopes (Fig. 16) whereas RSL are found on slopes facing the equator, sometimes associated with small channels or “gullies” (Ojha *et al.*, 2014)—although the pole-facing preference for gullies is less pronounced at higher southern latitudes (e.g., Balme *et al.*, 2006) and for the rarer, likely older gullies in the northern hemisphere (Heldmann *et al.*, 2007).

Many gullies were inferred to be relatively young (probably <1 Ma) based on their low crater densities and stratigraphic relationships with other landforms (Malin and Edgett, 2000; Schon *et al.*, 2009), but only in recent years following the first SR-SAG report (2006) has present-day gully activity been observed directly (Malin *et al.*, 2006). In some cases, images spaced only a few months apart have allowed determination of the season in which such activity occurred, providing new insights into gully evolution processes in the modern climate (Diniaga *et al.*, 2010; Dundas *et al.*, 2010; 2012; 2014b).

[Table 9 about here]

The morphologic, geographic and age ranges spanned by martian gullies suggest division into a few categories, to be separately evaluated. Here we use a taxonomy (Table 9) organized by Special Regions implications, not by gully morphology. By definition, the gullies of Taxon 4 (typically small, ~1–20 m wide) have a distribution equivalent to that of RSL, discussed in Section 4.1 above. However, these geomorphic gullies could endure longer than the seasonal RSL darkening, so it is feasible that some slopes dissected by such gullies have hosted RSL activity in the geologically recent past, and could be reactivated in the future—even if no RSL have been directly observed to date. Meter-scale gullies are resolvable only by HiRISE, so its spatial coverage, to date, sets the limits of our ability to map these potential Special Regions.

[Figure 17 About Here]

#### 4.2.1 Gully Type/Taxon 1

As of this writing, nearly 40 different bedrock-incised gully sites have shown unambiguous activity observed by Mars-orbiting spacecraft, with an additional 20 active sites on dunes or other sandy slopes (Dundas *et al.*, 2014b). All but two are in the southern hemisphere, with latitudes ranging from 29 to 72° (Fig. 17), although equatorial gullies have not yet been comprehensively surveyed. Activity includes topographic changes in the gully alcoves, channels, and aprons, with new sinuous channels being carved and volumetrically significant sediment being deposited in fans (Dundas *et al.*, 2012, 2014b). In all cases in which the

seasonality of this activity is constrained, it took place in the winter or early spring, at times and places with CO<sub>2</sub> frost present on the surface. This implies temperatures well below the lowest known eutectic for any H<sub>2</sub>O brine. These observations are consistent with models of gully formation driven by seasonal CO<sub>2</sub> frost activity, and inconsistent with liquid water playing an active role.

[Finding 4-2 Here]

While gullies for which activity has not yet been observed may have formed via similar processes, it is also possible that some of the erosion in these gullies may have been accomplished by liquid water. Indeed, while the freshest martian gullies have topographic profiles consistent with dry processes, the older and more degraded gullies appear more consistent with fluid involvement (Kolb *et al.*, 2010). Such liquid could have originated through the melting of surficial ice deposits that were laid down in the last glacial period, which culminated a few hundred thousand years ago (e.g., Schon and Head, 2011). Their potential for reactivation during the next 500 years depends on their access to water, and to sufficiently warm conditions to melt it, within that time period. With no direct evidence for shallow groundwater aquifers that might be accessed by these gullies (Section 4.4 below), we focus on the availability of residual ice that has not yet melted, dividing warmer gully sites on Mars into those that appear to have such ice vs. those that do not.

#### 4.2.2 Gully Type/Taxon 2

While temperatures are generally low in regions where ice is preserved today in the shallow subsurface, local microenvironments may experience warmer conditions. To be considered Special Regions, gully fluids would also require sufficiently high water activity (Section 3.2). This is difficult to constrain from available data, but there is currently no evidence that gully flows involve highly saline fluids. Although Malin *et al.* (2006) initially suggested that newly formed bright deposits in some gullies might be salt-rich, orbital spectroscopy has identified no salts in these freshly exposed materials (McEwen *et al.*, 2007; Nunez *et al.*, 2013). Their brightness may instead result from a finer average grain size compared to the surrounding slope, as observed in morphologically similar water-driven flows in the Atacama Desert (Heldmann *et al.*, 2010). However, neutral and dark deposits have also been observed (Dundas *et al.*, 2010; 2012; 2014 a or b), so it may be that deposit brightness reflects nothing more than the source area lithology. The runout distances of martian gullies has been argued to provide further evidence for relatively salt-poor fluids (Heldmann *et al.*, 2005), and in any case there is no reason to anticipate a high salt concentration in the ice-rich deposits that would source these potential gully flows.

[Finding 4-3 Here]

#### 4.2.3 Gully Type/Taxon 3

Gullies in warmer, ice-poor regions of Mars and not associated with RSL are generally not active today. A few possible instances of near-equatorial gully

activity appear to be consistent with dry mass wasting processes on steep slopes. With no ice or other apparent source of water for these gullies, they are judged to have a minimal risk of liquid water during the next 500 years (Fig. 18).

[Figure 18 About Here]

### **4.3 Recent craters that are still warm**

The formation of impact craters is associated with considerable heating of materials adjacent to the impact site. Studies of terrestrial impact craters reveal that many of these structures produced hydrothermal systems that persisted for extended periods of time following crater formation (Newsom, 1980; Osinski *et al.*, 2013) and where microbes were able to establish colonies during the active hydrothermal stage (Lindgren *et al.*, 2010; Ivarsson *et al.*, 2013). A variety of interior and ejecta morphologies associated with martian impact craters are interpreted as due to interaction with crustal volatiles (Barlow, 2010). Therefore martian impact craters must be investigated as potential Special Regions due to the possibility of associated hydrothermal systems (Pope *et al.*, 2006; Schulze-Makuch *et al.*, 2007).

Studies of terrestrial impact craters suggest that hydrothermal systems can be produced during the formation of complex craters (diameters > 2-4 km on Earth). Osinski *et al.* (2013) identify six main locations where hydrothermal deposits have been found to form in terrestrial craters: within ejecta deposits, along the crater rim, in the crater interior within impact melt rocks and melt-bearing

breccias, in both the interior and along the outer margins of central uplifts, and in post-impact crater lake sediments. The duration of active hydrothermal systems is proportional to the impact energy and thus the crater size—hydrothermal activity persists for greater periods in larger craters.

A growing body of evidence supports the idea that impact-induced hydrothermal systems also exist on Mars in association with many complex craters (diameters > 5-10 km) (Newsom *et al.*, 2001; Cockell *et al.*, 2003; Schwenger *et al.*, 2012). Orbital observations of surface mineralogy reveal that Noachian-aged craters often display hydrated silicate minerals, which could have formed from sustained hydrothermal activity (Poulet *et al.*, 2005; Mustard *et al.*, 2008; Schwenger and Kring, 2009; Carter *et al.*, 2010), although pre-impact or other impact-related formation mechanisms such as devitrification, autometamorphism, and alteration of impact-damaged materials have been suggested (Tornabene *et al.*, 2013). The Noachian Period (>3.85 Ga (Werner and Tanaka, 2011)) was characterized by warmer surface conditions and abundant liquid water on and near the martian surface—according to numerical modeling, these conditions would have allowed hydrothermal systems to remain active in crater central peaks and walls for  $10^3$ - $10^7$  years and for  $\sim 10^2$  years within ejecta deposits, depending on crater size (Rathbun and Squyres, 2002; Abramov and Kring, 2005; Ivanov and Pierazzo, 2011).

[Fig 19 About Here]

As Mars transitioned into colder, drier conditions associated with the Hesperian (~3.40-3.74 Ga) and Amazonian (~3.40 Ga to present) Periods (Werner and Tanaka, 2011), the evidence for minerals produced by impact-induced hydrothermal systems becomes less clear. Three Hesperian-aged craters—42-km-diameter Toro (17.0°N, 71.8°E) (Marzo *et al.*, 2010), 45-km-diameter Majuro (33°S, 84°E) (Mangold *et al.*, 2012), and 78-km-diameter Ritchey (28.5°S, 51°W) (Sun and Milliken, 2014)—expose evidence of hydrothermal minerals, including Fe/Mg phyllosilicates and opaline silica. However, some have argued that these aqueous minerals are simply exposures of Noachian-aged altered rocks that have been excavated from depth by the impact process (e.g., Ehlmann *et al.*, 2009; Fairén *et al.*, 2010). Fluvial landforms associated with large fresh craters such as 27.2-km-diameter Tooting crater (23.17°N, 152.17°E) (Morris *et al.*, 2010), 58.5-km-diameter Mojave crater (7.6°N, 32.6°E) (Fig. 19) (Williams and Malin, 2008; Goddard *et al.*, 2014), 125 x 150-km-diameter Hale crater (Jones *et al.*, 2011a; El-Maarry *et al.*, 2013), and several other Late Hesperian to Middle Amazonian-aged craters ranging between 12 and 110 km in diameter (Mangold, 2012; Goddard *et al.*, 2014) indicate that liquid water is produced during large impacts even under the present climatic conditions. Further evidence of interactions between target volatiles and post-Noachian impact craters is observed in pitted materials within crater cavities and ejecta blankets, which are proposed to represent degassing features from interactions of hot impact melt with crustal water (Tornabene *et al.*,

2012; Boyce *et al.*, 2012). Thus the conditions under which hydrothermal systems can be produced do appear to be met under current climatic conditions, although the intensity and duration of these systems are lower than was the case during the Noachian (Barnhart *et al.*, 2010).

[Figure 20 About Here]

Fig. 20 shows a summary of the duration of hydrothermal activity as a function of crater diameter based on results from numerical simulations of Newsom *et al.* (2001), Rathbun and Squyres (2002), Abramov and Kring (2005), and Barnhart *et al.* (2010) for craters ranging in diameter from 7 km to 200 km. Plotting craters as a function of their age and diameter on this graph allows determination of whether these craters may still retain active hydrothermal systems. Although orbiting spacecraft have confirmed the formation of over 400 new impact craters on the martian surface in the past few decades (Daubar *et al.*, 2013; 2014), none of these craters is large enough to have produced a hydrothermal system (i.e., all are much smaller than the 5-20 km diameter size necessary to initiate and sustain hydrothermal activity (Schwenzer *et al.*, 2012)). Determining ages of craters that have not formed during the ~40 years of martian orbiting spacecraft observations relies on the use of superposed crater density analysis, but can be fraught with error due to modification and secondary crater contamination issues.

Nevertheless, we have considered the possibility of active hydrothermal systems for three relatively young and large craters on Mars: 27.2-km-diameter Tooting crater ( $3 \times 10^6$  years old; Mougini-Mark and Boyce, 2012), 58.5-km-diameter Mojave crater ( $10^6$  to  $5 \times 10^6$  yrs; Werner *et al.*, 2014), and 125 x 150-km-diameter Hale crater ( $\sim 10^9$  yrs; El-Maarry *et al.*, 2013). These three craters are plotted on Fig. 20 and all fall above the line for the maximum sustained lifetime of hydrothermal systems for craters of their size according to the numerical simulations. Thus, although crater formation ages are highly uncertain, we have not been able to determine that any existing craters reported in the literature have the combination of size and youthfulness necessary for impact-caused hydrothermal activity to persist to the present. We therefore conclude that currently, the probability of extant hydrothermal systems in existing martian impact craters is low, and none define a Special Region in this way.

[Finding 4-4 Here]

#### **4.4 Groundwater**

Based on an estimate of the minimum volume of water required to erode the martian outflow channels and the likely subsurface extent of their original source regions, Carr (1986, 1996) concluded that, at the peak of outflow channel formation ( $\sim 3$  to 3.5 Ga, Tanaka, 1986; Hartmann and Neukum, 2001), Mars possessed a planetary inventory of water equal to a global equivalent layer  $\sim 0.5$ –1 km deep. Because this time postdates the period ( $>4$  Ga) when the most

efficient mechanisms of water loss were thought to be active, it is expected that virtually all of this inventory (in excess of the ~5% visible in the polar layered deposits) survives today in two thermally distinct subsurface reservoirs: (1) as ground ice within perennially frozen ground (known as the cryosphere) that extends from the near-surface down to depths of a least several kilometers in polar regions, and (2) as deep groundwater located beneath the cryosphere, where radiogenic heating is expected to increase lithospheric temperatures above the freezing point (Carr, 1979, 1996; Rossbacher and Judson, 1981; Kuzmin, 1983; Clifford, 1993; Clifford et al., 2010). Hydrous minerals in altered sections of the crust may be another important reservoir (Mustard et al., 2008; Ehlmann and Edwards, 2014).

Because the cryosphere is a natural cold trap for subsurface water, the survival of groundwater to the present day depends on the relative size of the planet's total inventory of water with respect to the storage potential of the cryosphere (Clifford, 1993; Clifford *et al.*, 2010). If the inventory of H<sub>2</sub>O exceeds the pore volume of the cryosphere, then the excess will be stored as groundwater, saturating the lowermost porous regions of the crust. However, if the subsurface inventory of H<sub>2</sub>O is less than the pore volume of the cryosphere, then all of the planet's original inventory of water may now be cold trapped within the cryosphere except where groundwater may be transiently produced by thermal disturbances of the crust, such as impacts, volcanism and climate change.

The depth of the martian cryosphere is determined by the latitudinal variation of mean annual surface temperature, the potential presence of freezing-point depressing salts, the thermal conductivity of the crust, and the planet's mean geothermal heat flow. Given reasonable estimates of these properties, the thickness of the cryosphere is estimated to vary from ~5 km at the equator to ~15 km at the poles, with natural variations in the values of these properties resulting in local differences of as much as +/-50% (Clifford *et al.*, 2010).

[Figure 21 About Here]

The most persuasive evidence for the past presence of groundwater on Mars is provided by the martian outflow channels—features, resembling dry terrestrial river beds, which emanate from isolated fractures or regions of collapsed and disrupted terrain, that appear to have been carved by the catastrophic discharge of groundwater (Carr, 1979; Baker *et al.*, 1992). While the occurrence of outflow channel activity appears to have spanned much of martian geologic time (Tanaka, 1986; Baker *et al.*, 1992; Carr, 1996), it is the evidence for geologically recent activity (~2 Ma – 1 Ga) in Mangala Valles (Basilevsky *et al.*, 2009), Kasei Valles and Echus Chasma (Chapman *et al.*, 2010; Neukum *et al.*, 2010), Athabasca Vallis (Fig. 21), Marte Vallis and the Cerberus plains (Hartmann and Berman, 2000; Burr *et al.*, 2002; Plescia, 2003) that provides the most compelling argument for the survival of groundwater to the present day. A counter argument

is that in all of these cases listed above the crater counts indicating young ages date lava flows that post-date channel formation (McEwen *et al.*, 2012).

[Finding 4-5 Here]

The detection of deep groundwater on Mars is a technically challenging task. This challenge motivated the flight of the Mars Advanced Radar for Subsurface and Ionospheric Sounding (MARSIS) orbital radar onboard ESA's MEX spacecraft, and the Shallow Radar (SHARAD) orbital radar sounder on NASA's MRO. MARSIS and SHARAD operate in a similar way, by emitting a radar pulse towards the surface and detecting the reflections caused when that pulse encounters interfaces between two materials of differing dielectric properties, among the greatest being the contrast between liquid water and dry or frozen rock.

MARSIS operates at frequencies of ~2–5 MHz, giving it a theoretical ability to sound the martian subsurface to depths of ~3–5 km under optimal conditions (Picardi *et al.*, 2004). In practice, MARSIS has achieved this level of sounding performance only in low-dielectric loss environments, such as the ice-rich polar layered deposits (PLD) (Plaut *et al.*, 2007), to < 1 km to the base of the south polar Dorsa Argentea Formation (Plaut *et al.*, 2007b), and several km depth in the Medusa Fossae Formation, whose radar propagation characteristics are consistent with a composition ranging from a dry, high-porosity pyroclastic deposit to an ice-rich sedimentary deposit, potentially formed by the redistribution of polar volatiles at times of high obliquity (Watters *et al.*, 2007).

However, in lithic environments, the absence of radar reflections at depths below ~200-300 m, whether from structural, stratigraphic or water-related interfaces, suggests that the martian subsurface is strongly attenuating—providing no insight regarding the presence of groundwater at greater depths (Clifford *et al.*, 2010). And, at shallower depths, there is no indication of the presence of groundwater anywhere on the planet, at least at the spatial coverage (nearly 100% complete at horizontal track spacing <30 km) and horizontal and vertical resolution of MARSIS.

With a 20 MHz operating frequency and 10-MHz bandwidth, the SHARAD orbital radar sounder is capable of an order of magnitude improvement in spatial resolution over MARSIS, but to a frequency-limited maximum sounding depth of ~2 km under ideal (i.e., low dielectric loss) conditions. SHARAD has sounded to such depths in the PLD (Phillips *et al.*, 2008) and in km-thick ice-rich LDAs that are found at the base of scarps at high and temperate latitudes (Plaut *et al.*, 2009). However, like MARSIS, it has found no evidence of groundwater within the top ~200 m of the subsurface anywhere on the planet, although full global reconnaissance is not yet complete. This includes any shallow reservoir of liquid water potentially associated with the martian gullies, which should be clearly visible in the orbital radar data.

[Finding 4-6 here]

The above results do not constrain the existence of diurnally or seasonally active near-surface brines, which, if they occur, almost certainly do so over depths no greater than the top several meters (or, more likely, the top ~25 cm (Chevrier and Rivera-Valentin, 2012; but see Clifford *et al.*, 2010)). Such features would fall well below the minimum vertical resolution limit of either MARSIS or SHARAD (~100 m and ~10 m, respectively).

[Finding 4-7 here]

#### **4.5 Slope streaks**

Slope streaks are found on steep, bright, dust-mantled slopes, mostly equatorial (Sullivan *et al.*, 2001; Aharonson *et al.*, 2003; Baratoux *et al.*, 2006; see Fig. 22). They are actively forming, and fade over time periods of decades (Schorghofer *et al.*, 2007; Bergonio *et al.*, 2013). They form as relatively dark features, but may brighten over time into relatively bright streaks. No seasonality has been detected (Schorghofer and King 2011), and no incremental growth has been reported, so they do not have the temporal behavior of RSL. Where incidence angles are high, HiRISE images show that a thin surface layer has been removed to create each streak (Chuang *et al.*, 2007; Phillips *et al.*, 2007). Most workers have interpreted these as dry dust avalanches, but alternative wet interpretations for some of these features have also been published (Ferris *et al.*, 2002; Miyamoto *et al.*, 2004; Kreslavsky and Head, 2009; Mushkin *et al.*, 2010).

[Figure 22 About Here]

The previous SR-SAG study (Beaty *et al.*, 2006) did not consider slope streaks in general to be potential Special Regions, and the COSPAR Colloquium (Kminek *et al.*, 2010) suggested they be evaluated on a case-by-case basis. No new results have encouraged an alternative interpretation of these features at this time.

[Finding 4-8 Here]

[Figure 23 About Here]

#### **4.6 Polar dark dune streaks**

A distinct class of active martian slope features occurs on dunes in both the north and south polar regions. While seasonal dark spots were identified on polar dune surfaces with MGS Mars Orbiter Camera (MOC) images, it was HiRISE that first revealed narrower linear or branching streaks extending downslope from these spots (Fig. 23), first in the southern high latitudes ( $\sim 54\text{--}72^\circ$ ; Kereszturi *et al.*, 2009) and later in the north ( $\sim 77\text{--}84^\circ$ ; Kereszturi *et al.*, 2010). These features appear and grow, extending farther downslope as the regional temperatures slowly rise from their wintertime low at the CO<sub>2</sub> frost point ( $\sim 150$  K). The streaks are up to a few meters across and extend tens of meters downslope, most commonly along the dune slipfaces. Their relatively dark appearance is at least partly due to the contrast of dark dune sand, revealed in the spots and streaks, relative to the CO<sub>2</sub> frost-covered surrounding surface; however, wetting has also been proposed as a possible darkening mechanism (Kereszturi *et al.*, 2010). The

streaks are no longer visible in summertime once defrosting is complete, and do not appear in exactly the same spots during subsequent years.

Möhlmann and Kereszturi (2010) argued that the streak morphologies and growth rates are consistent with viscous liquid flows, wherein the liquid is hypothesized to be concentrated brine (Kereszturi *et al.*, 2011). However, even the most extreme known brines require temperatures above ~200 K, which are unlikely when CO<sub>2</sub> frost still largely covers the adjacent dune surfaces, as confirmed by CRISM observations during the season of streak activity (Pommerol *et al.*, 2013a). An alternative hypothesis is that springtime CO<sub>2</sub> sublimation and gas flow initiate gravity-driven mass wasting of sand and ice down the dune slipfaces (Hansen *et al.*, 2011, 2013; Portyankina *et al.*, 2013), forming the dark streaks and the small gullies with which they associate in some cases (Fig. 23). Similar streaks have been observed to flow over presumed CO<sub>2</sub> frost within gully channels at a temperature < 150 K (Dundas *et al.*, 2012). Active dust avalanche clouds have also been observed in association with the streak-forming activity, supporting the mass wasting hypothesis (Hansen *et al.*, 2011). Even if eutectic brines are present in some of these locations, their temperatures would be far below the limits for demonstrated growth of terrestrial microorganisms (Section 3.1).

[Finding 4-9 Here]

#### **4.7 Thermal zones**

The ODY Thermal Emission Imaging System (THEMIS) infrared imager (~100 m/pixel) has been utilized to search for thermal anomalies associated with either near-surface magmatic activity or with surface cooling due to the evaporation or sublimation of sub-surface water or ice. These searches have been implemented using two methods—the first uses automated detection algorithms to identify pixel-scale temperature anomalies that are above a specified threshold. The second technique uses image-to-image differences to search for time-variable surface temperatures that might be indicative of varying sub-surface heat sources or sinks (Christensen *et al.*, 2008). Unfortunately there are complications associated with both techniques. In particular, there are significant spatial variations in the nighttime temperatures that are due to local variations in thermal inertia (particle size, rock abundance, and induration) and local slopes and fissures (Christensen *et al.*, 2005). This detailed temperature variability renders detection of temperature “anomalies” difficult, especially considering that even substantial sub-surface magmatic heat would be greatly attenuated at the surface. The more promising technique of comparing temperatures over time has been complicated by the continually changing local time of the ODY orbit. As a result, images taken at the same season on different years typically have different local times, making it difficult to directly compare year-to-year images to search for long-term internal heat changes (Christensen *et al.*, 2008). Previous observing conditions have therefore not been optimal.

Future surveys from a modified orbit will be undertaken, allowing the possibility of detecting areas that are anomalously warm in the future, although from a less advantageous orbital position. Upon detection of such a zone, independent assessments could then be made to determine whether the zone may also have higher concentrations of water vapor, or other forms of H<sub>2</sub>O. An approach that is being developed corrects for local time and season differences between images using a thermal model, but getting a robust thermal model that is accurate to the level required (1-3 K) is challenging. This work will continue, but to date there is no conclusive evidence for near-surface heat sources or sinks.

[Finding 4-10 Here]

#### **4.8 Caves**

By contrast with the surface, martian caves can provide protection against a number of challenges to the survival of Earth organisms—in particular UV and other radiation, and potentially from atmospheric conditions (e.g., solar-influenced dryness) as well. On Mars, special geomorphic regions may include caves in both volcanic terrains and other lithologies (e.g., evaporite basins or ice in polar terrains), and rock shelter overhangs in canyon & arroyo walls and scarps. Many and varied examples of each of these types of geologic features are present on Earth in a globally distributed fashion, and are present in almost every rock type present on the planet including ice and granite (e.g., Giggenback, 1976; Vidal-Romani and Vaqueiro Rodriguez, 2007), unconsolidated materials like

clays and other sediments (Clausen, 1970; Rogers, 1981; Davis, 1999; Halliday, 2004; BGS, 2011; Mud Caves List 2014) or even volcanic tuff (Parker *et al.*, 1964). A plethora of formation mechanisms are involved in this richness of subsurface crustal features (e.g., Klimchouk *et al.*, 2000; Ford *et al.*, 2007; Palmer, 2007; Kempe 2009, and many others) including even the role of microorganisms in cave enlargement (Summers-Engel *et al.*, 2004; Boston *et al.*, 2004; 2009).

To date, Mars mission imaging has yielded views of vertical pits or shafts of various sizes and descriptions in volcanic terrains that may be associated with some form of extensional tectonics, collapse of material into an emptied magma chamber, or other processes (Wyrick *et al.*, 2004; Cushing *et al.*, 2007; Smart *et al.*, 2011; Cushing, 2012; Halliday *et al.*, 2012). Caves on Mars were speculated about before they were identified (e.g., Grin *et al.*, 1998; 1999) and chains of collapse pits are now visible in many locations on Mars and interpreted as possible lava tubes, sinuous rilles, or other volcanic subterranean features (Boston, 2004; Cabrol *et al.*, 2009) See Fig. 24. Such features appear to be a byproduct of lava flows or dikes as they are here on Earth, and these can be made by a variety of mechanisms (Kempe, 2009; 2006). Methods to refine remote detection of such features are being undertaken (e.g., Cushing, 2007; 2012; Wynne *et al.*, 2008).

[Figure 24 About Here]

Besides volcanic caves and related features, the potential exists for caves in other lithologies on Mars. On Earth caves are common in soluble evaporites in aridlands where periodic moisture occurs from either precipitation or groundwater sources (Klimchouk *et al.*, 1996). Small scale surficial and cavernous karstification in evaporite terrains has been studied (e.g., Stafford *et al.*, 2008). Evidence of evaporite deposits on Mars and in Mars-derived meteorites, including carbonates and sulfates, has been reported (Bibring *et al.*, 2005; Gendrin *et al.*, 2005; Bridges and Grady, 1999; Morris *et al.*, 2010;) perhaps occurring in large basins (e.g., Ruff *et al.*, 2014). A type of catastrophic speleogenesis of cavities in evaporite facies as a result of meteorite impact has been suggested (Boston *et al.*, 2006). While the potential exists, to date no such specific non-volcanic subterranean features have been identified in imaging data.

Clearly, from the perspective of planetary protection, geomorphic features with natural openings into the subsurface could potentially be contaminated by spacecraft or spacecraft parts should they accidentally enter in the course of entry, descent, and landing (EDL) or while roaming the surface. Thus, the degree of enhanced habitability potential of such environments is of interest. On Earth, these cave and other subterranean features offer protected habitats for organisms that are more benign in a variety of ways than surface environments. Typically, even for shallow caves, the interior conditions are drastically different environments for microbial life from the immediately overlying surface

environment (e.g., Boston *et al.*, 2001; 2009; Northup and Lavoie, 2001; Leveille & Datta, 2010). Higher moisture, virtually no temperature variability, and protection from sunlight are all benefits of the subsurface lifestyle. The degree of enhanced habitability of subsurface terrain on Mars is unclear, however a major factor could be protection from ionizing radiation (Boston, 2010). Subsurface terrains on Mars may or may not house indigenous Martian life, but if they are of a higher quality of habitability, then that must be taken into account when assessing the potential for contamination by Earth organisms.

Caves with natural openings include most lava tubes, pit crater shafts, tumuli, rock shelters in cliff faces of varying lithologies, and dissolutional caves whose openings are typically created by subsequent geological processes, e.g., canyon incision. Such open or partially open caves are capable of being contaminated by spacecraft. In truth, because dissolutional caves are created on Earth typically in the vicinity of the water table, most have no natural openings and are relatively closed systems until they are breached by other geological processes (e.g., canyon incision, tectonic motions, etc.). Such closed caves on Mars are exceedingly unlikely to be contaminated by spacecraft barring the unlikely of a direct hit that breaches such a cavity. Thus, the cavities of concern are those that we have some chance of seeing with orbital assets.

[Finding 4-11 Here]

[FIG. 25 goes here somewhere.]

#### **4.9 Shallow subsurface conditions**

Ground temperatures are a primary driver for defining ice stability, water transport, water phase partitioning, and activity within the regolith (e.g., Leighton and Murray, 1966; Paige, 1992; Mellon *et al.*, 2004). Surface temperatures oscillate diurnally and seasonally, propagating into the subsurface with an amplitude that diminishes exponentially with depth (e.g., Fig. 25a). The presence of high-thermal-inertia ice, at depth, will act to wick heat from the shallower layers and greatly reduce the peak temperatures that occur within the ice (Fig. 25b).

Ground ice stability occurs when the annual mean vapor density over ice in the soil pore space, integrated over these seasonal cycles, equals that of the atmosphere (Mellon and Jakosky, 1993). At depths where the mean vapor density exceeds that of the atmosphere, ice will sublime and be diffusively lost. Likewise at depths where the vapor density of the atmosphere exceeds that in the soil, water will diffuse down and condense. On time-scales shorter than orbital changes and climate oscillations, this depth of diffusive equilibrium is maintained, tracking those changes (Mellon and Jakosky, 1995). Departures from the mean may occur diurnally and seasonally in the subsurface and atmosphere, but these changes are largely damped by the slower diffusive time-scales affecting the subsurface (Mellon and Jakosky, 1993; Mellon *et al.*, 2004).

Diurnal and seasonal temperature oscillations in the soil, and the slower diffusive time scales, may allow water vapor in the pore space to either build up or be depleted from time-to-time, relative to the atmospheric conditions. Thus the water activity in the pore space is not always equivalent to that of the atmosphere. The magnitude of this departure is largely unexamined, and will depend on several factors, including the thermophysical properties and porous structure of the soil, and its geographic location on Mars.

Fig. 26 illustrates seasonal differences in temperature and relative humidity as would be anticipated (and during the mission were partially experienced) at the PHX landing site, with over 40 K difference in the temperature ranges experienced in winter vs. summer at the site. The low amount of water in the atmosphere of Mars results in a very low relative humidity at the site when the temperatures approach the lower temperature limit for microbial cell division (255 K).

[FIG. 26 goes here somewhere]

[Finding 4-12 Here]

#### **4.10 Significant of deliquescence in the Mars natural environment**

Many salts on Mars, particularly perchlorate and chloride compounds, are likely to be deliquescent, meaning they can form an aqueous (liquid water) salt solution (i.e., a brine) via absorption of atmospheric water vapor by the crystalline salt (Renno *et al.*, 2009; Xu *et al.*, 2009; Zorzano *et al.*, 2009; Davila *et al.*, 2010;

Gough *et al.*, 2011; Wang *et al.*, 2012; Nuding *et al.*, 2014). In order to understand if, when and where deliquescence may be occurring on Mars (either naturally or spacecraft-induced) and under what conditions the resulting aqueous solutions may persist, we need to understand the temperature and humidity threshold values for deliquescence for different salt compositions, as well as the kinetic factors that may affect aqueous phase formation and disappearance.

[Figure 27 About Here]

A stable aqueous solution will form via deliquescence when the atmospheric relative humidity (RH) at a given salt's surface is greater than or equal to the deliquescence relative humidity (DRH) of that salt. Fig. 27 shows the stability diagram of a deliquescent salt likely to exist on Mars, calcium perchlorate,  $\text{Ca}(\text{ClO}_4)_2$  (Nuding *et al.*, 2014). The blue lines (both light and dark) represent the DRH values of relevant hydration states as a function of temperature. Additionally, for a stable aqueous solution to exist the temperature must be greater than or equal to the eutectic temperature ( $T_E$ ) of a given salt (Renno *et al.*, 2009; Kossacki and Markiewicz, 2014). The  $T_E$  value for  $\text{Ca}(\text{ClO}_4)_2$  is represented by a square black symbol in Fig. 27 (~197 K in this case). Finally, if there is too much water vapor (or, too low of a temperature for a given amount of water vapor), the stable phase of water in the mixture is ice. Therefore, in order for a stable aqueous solution to exist, the saturation with respect to ice ( $S_{ice}$ ) of the system must be less than 1 (i.e., to the right of the black line in Fig. 27). This ice

saturation line is the only aqueous stability limit that is independent of salt composition. These boundary conditions surround the region of aqueous phase stability, which is the blue shaded area in Fig. 27. The aqueous stability region extends upwards beyond the maximum temperature plotted here, but these warmer temperatures are not relevant to Mars.

It is predicted that a stable aqueous phase will form whenever the temperature and RH conditions enter the stability region defined by the limits outlined above, and this stable liquid will remain as long as suitable conditions persist. When conditions become too cold and/or wet for the aqueous phase to be stable (lower left region of Fig. 27), ice is predicted to form. Similarly, when conditions become too dry for the aqueous phase to be stable (lower right region of Fig. 27), the solution is predicted to crystallize into a solid salt. Both of these liquid-to-solid phase transitions require an increase in thermodynamic order of the system. As a result, both freezing and salt recrystallization are often kinetically hindered. This kinetic inhibition may allow metastable aqueous phase (i.e.: a brine) to remain under conditions that are too cold or too dry for thermodynamically stable solutions. These metastable solutions are supersaturated when present under low RH conditions that concentrate the brine beyond the point at which solid salt should precipitate, and are supercooled when the brine is below the temperature at which ice should precipitate. The extent of supersaturation and supercooling that can occur has been experimentally measured in the case of some

relevant salts (e.g.,  $\text{NaClO}_4$ ,  $\text{Ca}(\text{ClO}_4)_2$  and  $\text{Mg}(\text{ClO}_4)_2$ ) (Gough *et al.*, 2011; Nuding *et al.*, 2014; Toner *et al.*, 2014). In general, however, aqueous phase metastability is hard to model and predict for many reasons, one of which is the dependence on external factors that are not clearly understood (presence of regolith, composition and concentration of dissolved compounds, etc.). These metastable effects should nevertheless be considered whenever the temperature or humidity are lowered around a brine, especially at low temperatures more relevant to Mars. All known metastable effects systematically favor the existence of the liquid phase.

[Figure 28 About Here]

The stability diagram in Fig. 27 is valid only for  $\text{Ca}(\text{ClO}_4)_2$ , which is just one of the deliquescent salts known to exist in the martian regolith. Sulfates, chlorides, and additional perchlorate species have been detected in situ by PHX and MSL (Hecht *et al.*, 2009; Glavin *et al.*, 2013; Kounaves *et al.*, 2014b) and these salts behave differently with respect to formation of an aqueous phase. Fig. 28 depicts the variation in  $T_E$  of several ionic species that may exist on Mars. This line is the same equilibrium limit represented in Fig. 28 as a black line, and is independent of salt composition. It can be seen in Fig. 28 that martian salts may have a range of eutectic temperatures. However, the global distribution of salts is not known because many species in the regolith cannot be distinguished from orbit. Therefore, even if phase diagrams similar to Fig. 27 were available for all

Mars-relevant salts (which is not the case), the brine composition in the shallow martian subsurface cannot be predicted or mapped. This is reinforced by the fact that salts are often mixed together as parageneses, and the presence of an average ionic composition does not mean that all salts are homogeneously mixed.

However, because  $\text{Ca}(\text{ClO}_4)_2$  has been detected on Mars (Glavin *et al.*, 2013; Kounaves *et al.*, 2014b) and has the lowest  $T_E$  of any Mars-relevant salt (Pestova *et al.*, 2005), it is a useful case for this report to consider.

[Finding 4-13 Here]

#### *4.10.1 Deliquescence at the Phoenix and MSL landing sites*

For any location on Mars that contains deliquescent salts and at which we have measured the environmental conditions (temperature and relative humidity), the potential for brines to exist can be assessed, as can the habitability of the environment or microenvironment. Whenever temperature and relative humidity values lie within the aqueous stability region of a salt (for example, the blue shaded area in Fig. 27), a stable brine should exist (Chevrier *et al.*, 2009). At the Phoenix and MSL landing sites, instruments measured the temperature and relative humidity during multiple diurnal cycles (Zent *et al.*, 2010, Gómez-Elvira *et al.*, 2012). Other instruments onboard these spacecraft have confirmed the presence of  $\text{Ca}(\text{ClO}_4)_2$  in the regolith (Glavin *et al.*, 2013; Kounaves *et al.*, 2014b); therefore, Fig. 27 can be used to determine when an aqueous phase (i.e., a brine) is likely to exist at these locations. Plotted in Fig. 27 are three datasets

representing diurnal environmental conditions found at the landing sites of PHX (orange triangles), MSL (magenta and purple circles), and Viking 1 (red line) landing sites. The PHX data (68.2°N, 125.7°W) represents multiple sols throughout the mission binned and averaged into 1 hr intervals (Rivera-Valentin and Chevrier, 2014). All data were collected during the northern summer on Mars. The data from MSL (4.59°S, 137.44°E) represent two diurnal cycles (sols 15 and 17 of the mission,  $L_s = 157^\circ$ ) as measured by the Rover Environmental Monitoring Station, REMS, at the floor of Gale Crater (Gómez-Elvira *et al.*, 2012). There were no measurements of relative humidity at either Viking landing site, but the Viking 1 (22.5°N, 50.0°W) values plotted in Fig. 27 are from a numerical model used to predict conditions during sol 2 of the mission ( $L_s = 100^\circ$ ), constrained by the observed temperatures (Savijarvi, 1995). This Viking model is the only dataset that has local time of day associated with each (T, RH) data point, and several of these times are labeled in Fig. 27.

The results from PHX and MSL, as well as the modeled conditions at Viking 1, are similar in magnitude and behavior of diurnal RH and temperature variation. Comparing these datasets to the aqueous stability region of  $\text{Ca}(\text{ClO}_4)_2$ , it can be seen that the humidity and temperature at all of these locations are, for limited amounts of time, sufficient for the deliquescence of calcium perchlorate (possibly the most deliquescent salt on Mars) and thus formation of an aqueous salt solution (brine). These periods of liquid stability likely occur in the late morning and in the

evening (Nuding *et al.*, 2014). Although during most of each sol represented here conditions are too dry or cold for a liquid phase to exist, metastable brines likely persist even after the environmental conditions suggest formation of water ice or solid salt should occur, according to thermodynamic equilibrium.

[Finding 4-14 Here]

#### *4.10.2 Limits on Deliquescence in Forming a Habitat*

Although deliquescence likely occurs at the locations considered here, it does so at a temperature ( $<-65^{\circ}\text{C}$ ) far below that needed for cell division ( $>-18^{\circ}\text{C}$ ). Additionally, the water activity ( $a_w$ ) of the solutions formed via  $\text{Ca}(\text{ClO}_4)_2$  deliquescence at martian temperatures is too low to be habitable (i.e., the brine is too concentrated). These temperature and relative humidity limits for Special Regions are represented by the green box in the upper left of Fig. 27. The environmental conditions, specifically surface and subsurface relative humidity, elsewhere on Mars are not known at this time. Based on the diurnal cycles shown in Fig. 27, however, there is limited variation in diurnal RH and temperature conditions with location or season. Because of the large difference between the conditions present when a liquid water phase exists and the conditions needed to qualify as a Special Region, it seems unlikely that natural deliquescence on Mars will result in formation of a Special Region. Supercooling and supersaturation may result in aqueous solutions persisting under even lower temperature and lower RH (hence lower  $a_w$ ) conditions than is thermodynamically stable ( Renno

*et al.*, 2009; Gough *et al.*, 2011; Nuding *et al.*, 2014; Toner *et al.*, 2014). While this longer duration of liquid is interesting, these metastable liquids are even less habitable than stable brines formed on Mars, and therefore neither stable nor metastable brines formed by natural deliquescence are thought to qualify as Special Regions.

[Finding 4-15 Here]

[FIG. 29 here somewhere]

#### **4.11 Contemporary snow deposition**

Having brought a new observational capability to the surface of Mars, the LIDAR (light detection and ranging) on the PHX lander (Whiteway *et al.*, 2009) observed that water ice clouds form in the martian planetary boundary layer in the late summer, and grow large enough to precipitate significant distances through the atmosphere of Mars—and can reach the surface on occasion (in particular, during the early morning hours; Fig. 29). The PHX LIDAR demonstrated that these water ice crystals (i.e., snow) would be capable of reaching the ground. It is unknown how long that snow would last under daytime conditions, but it is expected to be a very short time. The melting of such snow has not been observed directly by any Mars spacecraft so far, although Viking 2 commonly observed frost (and its sublimation) at its Utopia Planitia landing site (Wall, 1981; Svitek and Murray, 1990). Melting is expected to be difficult because sublimation during the rise to the melting temperature is sufficient to remove most

frost, and the latent heat loss at the melting point dominates the thermal budget (Ingersoll, 1970; Hecht, 2002).

Given the circumstances, when snow falls on contemporary Mars it may regularly be missed because: 1) it falls principally in the dark of night and is therefore not seen by spacecraft imagers of any kind, nor by any other instrument with the resolution to see it; and 2) it does not last very long when it does fall, either sublimating away as a solid or by melting/boiling as the sun rises in the morning. If it chiefly sublimates, then it would have no consequence beyond that of the observed frost layers (which are quite thin; see Wall, 1981). If it melts/boils, it could provide a limited-lifetime Special Region on Mars, but leave behind atmospheric-interaction products and those due to UV-H<sub>2</sub>O-substrate interactions, including H<sub>2</sub>O<sub>2</sub> deposition and the buildup of other peroxide and perchlorate compounds. It should be possible for snow that falls to do so in a non-uniform manner, so that drifts or other phenomena might focus those effects on a particular area or areas.

[Finding 4-16 Here]

## **5. Considerations Related to Spacecraft-Induced Special Regions**

### **5.1 Characteristics of landing spacecraft**

A spacecraft that lands on Mars introduces a source of thermal energy foreign to the area at which it would be located. If H<sub>2</sub>O ice is present at its location, the ice or its surroundings could be warmed to above the threshold temperature at

which organisms could proliferate, thereby creating a Special Region. With sufficient heat and especially with a vapor barrier, not only high relative humidity but also liquid water could form. The specific composition of the ice, icy soil, or liquid are important because high concentrations of certain salts can lower the water activity below the critical level or cause chemical inhibition of growth (Sections 3.2, 3.3).

For these reasons, it would be important that each landing mission evaluate the potential for the presence of near-surface ice and the potential for the spacecraft to warm that ice sufficiently to create a Special Region, whether operating as expected or in an unplanned manner. The likelihood of the presence of near-surface H<sub>2</sub>O ice can be assessed based on landing latitude, the nature of the regolith, and whether there is evidence for or against shallow ice-containing regolith. While near-surface ground-ice by itself is not deemed to be Special, the heating of ice under specific circumstances (such as heat from a spacecraft Radioisotope Thermoelectric Generator (RTG) or from human-related surface activities) could produce near-surface liquid environments, which could be classified as Special Regions.

The extent of warming of the local region by the spacecraft requires consideration of nominal operating modes, failure modes, and thermal modeling of all scenarios.

#### *5.1.1 Spacecraft landing scenarios/modeling*

During terminal propulsive landing, large quantities of heat are generated by the firing of descent engines. For example, the Viking, PHX, and upcoming InSight (Interior Exploration using Seismic Investigations, Geodesy, and Heat Transport) mission all use(d) engine firings that continue down to the surface in order to accomplish soft landings on Mars. Likewise, the MSL rover Curiosity, the proposed Mars 2020 rover, and (possibly) the ExoMars rovers are deployed to the surface with descent engines firing several meters above the surface of the landing site. Pathfinder and MER used the air bag landing technique to avoid descent engines, although the bags were inflated with warm (and water-vapor-containing) gas. Whichever technique would be used, a thermal analysis would be necessary to assess the effects of the spacecraft-induced thermal anomaly should the site contain near-surface ice.

Once landed, spacecraft on Mars are typically perched on landing footpads or on rover wheels. In either case, the opportunity for direct transfer of heat by conduction would be limited. Although the footpads can be large, they typically are mounted to struts of titanium alloy, which has an inherently low thermal conductivity. The area of contact by wheels would be mainly determined by the compressibility of the soil combined with the downward pressure on each individual wheel. The typical martian regolith is fine grained which can provide a very significant amount of thermal insulation from the subsurface. Also, the low martian atmospheric pressure inhibits the transfer of heat by convection cells.

A reality of spacecraft design for operating in the cold environment of Mars would be that most component equipment that dissipates significant electrical energy as heat would be surrounded by built-in thermal insulation, whether as an individual box or by being located within a central thermally-controlled compartment. Exteriors can remain relatively cold. Often, however, peripheral mechanisms must be heated to condition lubricants and maximize mechanism lifetime. Wheel motors are an example requiring special consideration of the thermal imprint they may make on soil or ice-laden permafrost. Robot arm motors, including any located in the end-effector, may also be pre-conditioned by electrical heaters before being operated.

Additionally, there can be heat generated by mechanical action. Frictional forces are often necessitated by the sampling technique, generating additional heat beyond that due to operating the mechanism itself. Examples include the vibrating sieve on the Viking sampler, the rasp on the PHX sampling arm, the grinder on the Rock Abrasion Tool (RAT) on the MER rovers, or the drills on the MSL and planned ExoMars and proposed Mars 2020 rovers. If the target material is ice or contains ice, then a small but unavoidable Special Region may be created. Such Special Regions may be small, localized and very transitory in nature, however, and hence may be determined to not be a significant threat to the protection of the planet. Other temporary Special Regions might be created, for example, if the hot aeroshell, heatshield, backshell, or skycrane components land

on icy ground, or if large amounts of wheel slip or scuff occur over a short time in the event of becoming “stuck.”

Heat can also be transferred by “thermal radiation,” i.e., emission of infrared photons. Calculations by spacecraft thermal models routinely include this type of heat loss. Such models are chiefly aimed, however, at assuring that all spacecraft active components remain within their operating or at least their survival temperature ranges. For assessing the potential for creating a Special Region, these models must be extended to evaluate the effects of heat flux onto the local surface, as well as shadowing effects, etc. If ice is present in the surface, it will increase its rate of sublimation in response to the heating, but may not be able to do so at a rate that overcomes the latent heat of sublimation during the phase change, which could prevent the temperature from rising above the threshold temperature for proliferation of life. The lifetime of such a Special Region will depend on the volume of ice that is melted, compared to the sublimation rate of the remainder of the ice. If the temperature rises sufficiently to cause transition from ice to liquid H<sub>2</sub>O, then the liquid may begin to boil at a small additional increase in temperature due to the low pressure of the martian atmosphere. If boiling is initiated, the loss of H<sub>2</sub>O from the ice-reservoir will be much faster, and the Special Region may self-deplete rapidly and hence self-destruct.

Thermal analyses of the exterior radiating surfaces of the spacecraft must be considered, in addition to the heat transfer by appendages. In addition, transfer of

heat by advection, i.e., by wind, must also be evaluated to determine if it could be a significant factor in distributing heat more widely. Because conducted heat only flows in the direction of decreasing temperature, any spacecraft surfaces that are below the biological critical temperature are not of concern for creating a Special Region. Although they may be able to warm ice or even create liquid brine by deliquescence, the temperature of the ice or brine will be below the threshold temperature.

[Figure 30 about here]

#### *5.1.2 Non-nominal spacecraft landing scenarios–crashes*

In addition to evaluating the potential for creating a Special Region in an icy area due to nominal operation of the spacecraft or vehicle, consideration must be given to any credible failure mode that results in an off-nominal landing or operation that could increase the injection of thermal energy into an icy surface (Fig 30). An unintended hard landing would impart kinetic energy, most of which would be converted to heat, as well as direct transfer from the heat capacity of a potentially hot structure from atmospheric heating during an inadequately protected entry. “Breakup and Burnup” scenarios would provide the ability to model such anomalous events.

[Figure 31 about here]

[Figure 32 about here]

Of special concern are heat sources purposely provided by the energy of radioactive decay. Radioisotope heating units and especially the much larger RTGs would generate heat energy for several decades (cf., NASA, 2006). Normally these units would be at locations such that the transfer of heat to the ground would not be large, but this should be evaluated on a case-by-case basis. The more challenging analysis would be that of an anomaly that would allow an RTG or its radioactive component(s) to be released from spacecraft while still at a high velocity and hence with enough kinetic energy to become buried at some depth into the ground (Fig. 30). This would be germane to the issue of creating a Special Region for several reasons, including that the burial enhances the injection of thermal energy into the subsurface (minimizing the fraction lost to the atmosphere and space) and that the soil can act as a diffusion barrier to water vapor, allowing a high relative humidity microenvironment to develop, as well as retarding the rate of loss of H<sub>2</sub>O and hence prolonging the lifetime of the Special Region (Fig. 31). Detailed analyses must be made of the likelihood of the anomaly happening, and the sizes and durations of Special Regions formed by various scenarios (Fig. 32). Information on modeling impact burial was provided in the previous Special Regions Report (Beatty *et al.*, 2006).

[Finding 5-1 Here]

The current understanding about the existence of water ice in a variety of different areas on Mars is discussed below.

## 5.2 Tropical mountain glaciers

Mars in its history has been characterized by significant variations in its spin-axis/orbital elements (obliquity, eccentricity and precession) (Laskar *et al.*, 2004) and these variations have led to the redistribution of water currently in the polar ice deposits to lower latitudes to create ice ages, glaciers and their related deposits (e.g., Head *et al.*, 2003). Topographic and imaging data acquired by spacecraft have revolutionized our understanding of these deposits, providing detailed information that helps to characterize their key parameters (structure, morphology, slopes, elevations, morphometry, stratigraphic relationships, etc.). On the basis of these data, criteria have been developed to recognize additional non-polar ice-related deposits that might represent the glacial and periglacial record of these spin-axis excursions (Head *et al.*, 2010).

These data have revealed that the Amazonian era was characterized by a variety of ice-related deposits (Neukum *et al.*, 2004; Head and Marchant, 2008; Carr and Head, 2010) ranging from the pole to the equator in distribution. These include: 1) High- to mid-latitude mantles (Kreslavsky and Head, 1999; 2000; Mustard *et al.*, 2001; Milliken *et al.*, 2003; Head *et al.*, 2003) and polygonal patterned ground (e.g., Mangold, 2005; Levy *et al.*, 2010b); 2) Mid-latitude deposits such as LDA and lineated valley fill (LVF) (Lucchitta, 1981; 1984; Mangold, 2003; Pierce and Crown, 2003; Li *et al.*, 2005; Head *et al.*, 2005, 2006a; 2006b; 2010; Levy *et al.*, 2007; 2008; Morgan *et al.*, 2009; Baker *et al.*,

2010; Dickson *et al.*, 2008; 2010), Concentric Crater Fill (CCF) (Kreslavsky and Head, 2006; Levy *et al.*, 2009a, 2010a; Dickson *et al.*, 2012; Beach and Head, 2012; 2013), phantom LDA (Hauber *et al.*, 2008) and Pedestal Craters (Kadish and Head, 2011a; 2011b; Kadish *et al.*, 2009; 2010); and 3) Low-latitude Tropical Mountain Glaciers (TMG) (Head and Marchant, 2003; Head *et al.*, 2005; Shean *et al.*, 2005, 2007; Kadish *et al.*, 2008). General circulation models (e.g., Haberle *et al.*, 2003; Forget *et al.*, 2006; Madeleine *et al.*, 2009) and glacial flow models (e.g., Fastook *et al.*, 2008; 2011) illustrate the orbital parameters and atmospheric/surface conditions under which periods of non-polar glaciation are favored, and the resulting patterns of accumulation of snow and the flow of ice (Milliken *et al.*, 2003; Forget *et al.*, 2006; Madeleine *et al.*, 2009; Fastook *et al.*, 2008; Fastook *et al.*, 2011).

[Figure 33 about here]

Some of the largest of the non-polar ice-related deposits are seen in the equatorial regions of Mars in the form of Amazonian-aged TMG deposits (Fig. 33). Head and Marchant (2003) combined then-new data from the Mars Orbiter Laser Altimeter (MOLA) and images from the MOC on the MGS mission with field-based observations of the flow, surface morphology, and depositional history of polar glaciers in Antarctica (Marchant and Head, 2007). They showed that the multiple facies of an extensive fan-shaped deposit on the western flanks of Arsia Mons volcano (Zimbelman and Edgett, 1992) are consistent with

deposition from cold-based mountain glaciers, including drop moraines and sublimation till, and that some debris-covered glacier (DCG) deposits may still be underlain by a core of glacier ice. These surficial deposits provide compelling evidence that the western flank of Arsia Mons was occupied by an extensive (166,000 km<sup>2</sup>) tropical mountain glacial system accumulating on, and emerging from, the upper slopes of the volcano and spreading downslope to form a piedmont-like glacial fan. Scanlon et al. (2014) have documented evidence of Late Amazonian volcano-ice interactions, as eruptions from the flanks of Arsia continued during the period of glaciation, in some cases producing localized wet-based conditions and meltwater outflows. Shean *et al.* (2007) further mapped several high-elevation graben on the western flank of Arsia Mons that are interpreted as the source regions for late-stage, cold-based glaciers that overflowed graben walls, advanced tens to hundreds of kilometers downslope, experienced subsequent retreat, and left distinctive depositional features similar to those associated with cold-based glaciers in the Dry Valleys of Antarctica. These new observations and crater count data provided additional evidence for several periods of Late Amazonian tropical mountain glaciation within the past few 100 Myr. MOLA topography reveals that several lobate features interpreted as remnant debris-covered ice from the most recent phase of glaciation are presently hundreds of meters thick, suggesting the possibility of long-term, near-surface water ice survival in the equatorial regions of Mars.

A similar set of circumstances characterize the fan-shaped deposits on Pavonis Mons (Shean *et al.*, 2005; Forget *et al.*, 2006) with atmospheric deposition of water ice on the northwestern flanks of the Tharsis Montes during periods of high mean obliquity, leaving ice sheets for each of the Tharsis Montes glaciers attaining average thicknesses of ~1.6–2.4 km, values that are consistent with a cold-based glacial origin. The results of Shean *et al.* (2005) suggest that multiple phases of tropical mountain glaciation occurred on Mars within the past few hundred Myr (Kadish *et al.*, 2014) and that significant amounts of near-surface, equatorial ice may remain within the deposit today, as well as in the smaller Ascræus fan-shaped deposit. Accordingly, it should not be surprising that remnant debris-covered piedmont glacial deposits were proposed to explain features seen around the northwest flank of the Olympus Mons scarp by Milkovich *et al.* (2006). These features had previously been interpreted variously as landslide, pyroclastic, lava flow or glacial features but the advent of multiple high-resolution image and topography data sets permitted a new analysis. Basilevsky *et al.* (2005) analyzed High Resolution Stereo Camera images and topography and showed that the western part of the Olympus Mons edifice is composed not only of lavas but also of sedimentary and volcanic–sedimentary rocks consisting of dust, volcanic ash, and H<sub>2</sub>O ice that precipitated from the atmosphere. They concluded that glaciations seen along the western foot of Olympus Mons (e.g., Lucchitta, 1981; Milkovich *et al.*, 2006) also covered the

gentle upper slopes of the edifice, with possible remnant ice preserved today, protected from sublimation by a dust blanket.

[Finding 5-2 here]

### **5.3 Tropical and mid-latitude ice deposits**

Again, a major question is the location of any remaining surface and near-surface water ice, its origin, configuration and mode of occurrence and the depth to buried ice deposits. A more focused question is whether the ice resides at depths less than ~5 m from the surface. A range of deposits are thought to currently host buried ice, including DCG or LDAs, CCF, LVF, and potentially TMG. There are several pieces of evidence for the thickness of the debris cover and the depth to buried ice, including RMC; radar data; and models of CCF, LDA, and LVF. Several sources of data suggest that the debris thickness, and thus the depth to the buried ice, is at least 10-15 m.

[Figure 34 About Here]

#### *5.3.1 The distribution of “ring-mold craters”*

Ring-mold craters (Kress and Head, 2008; Fig. 34) are unique crater forms that have been interpreted to indicate penetration into a debris-layer covering buried ice and the partial excavation of the buried ice. Using the size-frequency distribution of smaller, bowl-shaped craters (interpreted to have penetrated only into the debris cover) and larger RMCs, an estimate of the thickness of the debris cover can be made (Fig. 34). For example, these data suggest that the thickness of

the current debris cover in CCF is about 15-20 m, much thinner than the total thickness of the often several km thick CCF (Kress and Head, 2008; Beach and Head, 2012; 2013). A set of relatively fresh ring-mold craters superposed on the Arsia and Pavonis Mons TMG deposits are interpreted to indicate that the impact events penetrated a veneer of sublimation lag and that buried remnant glacial ice lies at a depth of at least 16 m (Head and Weiss, 2014). Lobate debris aprons show a population of RMCs suggesting a 10-15 m depth to ice (Ostrach *et al.*, 2008). No RMC populations have been mapped with a diameter distribution that would suggest the presence of buried ice at depths shallower than ~10-15 m.

### *5.3.2 Depth to ice using MRO SHARAD data*

Where SHARAD data have resolved the lobate debris aprons (LDA) of Eastern Hellas and Deuteronilus Mensae (Holt *et al.*, 2008; Plaut *et al.*, 2009), the hundreds of meters of ice below the debris cover appears relatively debris-free, and the debris cover is interpreted to be of the order of 10-15 m thick. SHARAD has not yet detected buried ice in the residual TMG deposits (Campbell *et al.*, 2013).

### *5.3.3 Models of lobate debris apron emplacement*

These models (Fastook *et al.*, 2013) suggest that a debris thickness of the order 10-20 m is very realistic and plausible.

[Figure 35 about here.]

The distribution of various features indicative of tropical and mid-latitude ice deposits is shown in Fig. 35.

[Finding 5-3 Here]

[Finding 5-4 Here]

[Figure 36 about here.]

#### **5.4 Use of fresh impacts to infer ground ice**

Impact craters excavate to depths proportional to their diameters and therefore expose subsurface materials within these depths. Over 400 impact craters have formed over the past few decades during which orbiting spacecraft have been monitoring Mars (Daubar *et al.*, 2013; 2014). These new craters are typically identified by albedo changes in dust-covered regions of the planet as viewed in repeated orbiter observations. More than 25 such craters at mid- to high-latitudes have exposed bright materials in their interiors and ejecta blankets (Fig. 36; Byrne *et al.*, 2009a; Dundas *et al.*, 2014a). The craters range in size from 1.0 to 24 m in diameter and are estimated to be excavating material from depths of centimeters to a few meters. The bright materials fade and shrink in size over months to years, suggesting the high albedo material is exposed ice that sublimates away when exposed to the low-pressure martian surface conditions (Byrne *et al.*, 2009a; Dundas and Byrne, 2010; Kossacki *et al.*, 2011; Dundas *et al.*, 2014a). At one site, meter-sized ejecta blocks shrank or disappeared on a time scale of months to

years, suggesting that these blocks were excavated chunks of ice that also underwent sublimation.

[Figure 37 about here]

Twenty-four of the ice-exposed craters are found in the northern hemisphere between 39°N and 65°N, with only two thus far confirmed in the southern hemisphere (between 71°S and 74°S; Fig. 37). The apparent concentration of these new ice-exposing craters at northern latitudes is the result of the detection technique (due to the greater areal coverage of dust in the north) and is likely not a reflection of lack of near-surface ice in the southern mid- to high-latitudes. The shallowest craters with exposed ice are found to occur at the highest latitudes, consistent with thermal models that indicate ice is stable closer to the surface at higher latitudes (Mellon *et al.*, 2008). The largest nearby craters without icy deposits often have flat floors, suggesting excavation to the top of (but not into) a resistant subsurface layer that is interpreted as the ice table.

The rate at which the bright regions darken, together with some spectral results from CRISM, suggest that the ice is very clean (~1% regolith content) and not simply exposed pore ice (Dundas and Byrne, 2010; Reufer *et al.*, 2010; Cull *et al.*, 2012). Instead, the ice appears to be excess ice, which is ice that exceeds the dry soil pore space, although some of the clean ice could be from melted pore ice that ponded on the surface. Several possible origins have been proposed for the excess ice, including vapor deposition of ice in small spaces opened by cracking

and differential contraction (Fisher, 2005), frozen floodwaters, pingos, or buried glaciers (Mellon *et al.*, 2008), near-surface ice lenses from migration of thin films of liquid (Mellon *et al.*, 2009; Sizemore *et al.*, 2014), DCG (Plaut *et al.*, 2009), buried snow deposited during higher obliquity periods (Schorghofer and Forget, 2012), or hydrothermal circulation of brines in the near surface region (Travis *et al.*, 2013). No single model adequately explains all the observations of the icy craters, although migration of thin films of water is the best fit to the majority of observations (Dundas *et al.*, 2014a).

The direct detection of ice exposed by these new craters has pushed the distribution of near-surface ground-ice to lower latitudes in the northern hemisphere (down to 39°N) than what has been known previously from neutron spectrometer results (Feldman *et al.*, 2004). However, the presence of non-icy new impacts indicates that not all fresh craters excavate bright ice even in regions where near-surface ice is expected to be present (Dundas *et al.*, 2014a). Therefore near-surface ice may be more heterogeneously distributed than previously predicted based on neutron spectrometer results and periglacial landform distribution. Alternatively, some craters without bright ice may have exposed pore ice, which would quickly become indistinguishable from regolith (as observed at the PHX landing site; Mellon *et al.*, 2009). The southernmost extent of the icy impact craters in the northern hemisphere is greater than that indicated from the neutron spectrometer analysis and thus requires a long-term average atmospheric

water content that is moderately higher (~25 pr. microns) than the present value (Dundas *et al.*, 2014a), geographically- and temporally-varying atmospheric water vapor content due to obliquity variations (Chamberlain and Boynton, 2007), geographic concentration of water vapor near the surface (Zent *et al.*, 2010), or control of vapor pressure due to brines formed by deliquescent salts in the regolith (Cull *et al.*, 2010). In addition, inference of possible (or previous) near-surface ice in near-equatorial regions has been made based on interpretation of certain landforms (e.g., Balme and Gallagher, 2009), but the lack of bright deposits with the behavior of ice exposed in fresh craters at low latitudes suggests that ice is no longer present at these locations within the depths excavated by the craters.

[Finding 5-5 Here]

[Figure 38 About Here]

### **5.5 Use of polygonal ground to infer ground ice**

Polygonal patterned ground is a ubiquitous mid-to-high-latitude landform (Fig. 38). Terrestrial counterparts are well understood to form by repeated seasonal thermal-contraction cracking of cohesive ice-rich permafrost (Lachenbruch, 1962). They typically develop into visible rectilinear networks of troughs spaced meters to tens of meters apart, underlain by subsurface accumulations of material (soil and/or ice) which fall each year into millimeter-scale seasonal fractures. Although morphological details may vary, these landforms typically exhibit narrow size distributions, equiangular junctions, and

honeycomb-like patterns with a variety of topographic profiles depending on the evolution of subsurface ice. Martian forms are similar in scale and morphology (Mutch *et al.*, 1977; Mangold, 2005; Levy *et al.*, 2009a; Mellon *et al.*, 2009a) and inferred to form by the same process (Mellon, 1997).

The occurrence of polygonal patterns can be interpreted as indicating extensive long-lived ground ice, in the recent geological past or persisting today (e.g., Mangold, 2005; Arvidson *et al.*, 2008; Smith *et al.*, 2009). Polygons correspond to regions where gamma ray and neutron spectrometer data indicate abundant ground ice (Levy *et al.*, 2009b) and were examined in close detail at the PHX landing site, confirming the thermal contraction origin and present day activity (Mellon *et al.*, 2009a). Polygonal ground illustrates local-scale variability not resolved in other datasets (Mellon *et al.*, 2010; 2014).

Some polygonal landforms occur in equatorial regions, but are either clearly associated with volcanic flows, lava cooling, and volcanic deflation (e.g., Ryan and Christensen, 2012) or are associated with fractured bedrock, in which case they exhibit irregular size distribution, shape, and fracture density (Yoshikawa, 2003). These types of polygons are generally not considered to be related to ice-rich permafrost.

The occurrence of polygonal ground provides supporting evidence of the permafrost's ice-rich status. In addition, local scale variability may be discriminated through polygonal geomorphology that is not resolved by lower

resolution data sets, such as those from ODY THEMIS, or the even-lower resolutions of ODY High Resolution Neutron Spectrometer or Gamma Ray Spectrometer.

[Finding 5-6 Here]

### **5.6 Near surface ice stability, concentration, and distribution**

The global distribution of shallow ice-rich permafrost was previously examined as it relates to Special Regions (Beatty *et al.*, 2006). In the current martian climate, ground ice in the upper meters of the regolith has been predicted to be present in the middle- and high-latitude regions (Leighton and Murray, 1966). During the subsequent decades after this prediction there have been numerous studies examining aspects of ground-ice stability and refining this initial prediction. For example, studies have included effects of: global and seasonal atmospheric water measurements (Farmer and Doms, 1978; Chamberlain and Boynton, 2007); variability in soil properties (Paige, 1992; Mellon and Jakosky, 1993, Mellon *et al.*, 2004); orbitally-induced climate change (Fanale *et al.*, 1986; Mellon and Jakosky, 1995; Chamberlain and Boynton, 2007); and the observed distribution of surface slopes (Aharonson and Schorghofer, 2006). Observations by ODY of gamma rays and leakage neutrons emitted from the surface later confirmed the presence of subsurface ice (Boynton *et al.*, 2002; Feldman *et al.*, 2002; Mitrofanov *et al.*, 2002) in the geographic locations and at depths as were predicted (Boynton *et al.*, 2002; Mellon *et al.*, 2004; Prettyman *et*

al., 2004; Diez *et al.*, 2008; Feldman *et al.*, 2008). These findings illustrate that we understand ground-ice stability and that diffusive equilibrium between the subsurface and the atmosphere in the present climate is the fundamental controlling process. In addition, the concentration of ice in the shallow permafrost was observed to be highly variable on a regional (1000 km) scale ranging from soil-pore filling to ~90% by volume (Prettyman *et al.*, 2004).

Since the 2006 study, several new observations have shown the presence of ground ice either directly or indirectly. These results generally confirm that the geographic and depth distribution of ground ice agrees well with the predictions and that the distribution of ice is controlled primarily by diffusive equilibrium in the current climate (ground temperature and atmospheric humidity). The concentration of ice, however, in many locations and at many spatial scales, exceeds the predicted pore volume. This excess ice remains poorly understood and may indicate some role of liquid water in the modern martian climate (Mellon, 2012).

[Figure 39 about here.]

On May 25<sup>th</sup>, 2008 the Mars Scout mission PHX landed in the northern plains of Mars at 68.22°N 234.25°E in a region expected, based on theoretical and observational evidence, to be dominated by shallow ice (Arvidson *et al.*, 2008; Smith *et al.*, 2009). Phoenix confirmed that shallow ground ice persists in this terrain (Fig. 39), through direct excavation and the erosive action of the decent

thrusters (Mellon *et al.*, 2009b). This ice was also found at a depth (2–6 cm below the surface) that had been predicted assuming the ice is in equilibrium with the current climate (Mellon *et al.*, 2008). Ground ice at the PHX site was also shown to be highly variable in concentration from pore filling to ~99% pure (excess ice) over lateral scales of less than one meter (Mellon *et al.*, 2009b).

Seasonal condensation and sublimation of CO<sub>2</sub> frost at non-polar latitudes can be a sensitive indicator of the presence of shallow ground ice. Ground ice (like bedrock) exhibits a high thermal inertia relative to the uncemented dry permafrost that lies above it. If ice is proximal to the surface it can alter the seasonal temperatures, and cause CO<sub>2</sub> frost formation to be delayed and spring sublimation to occur earlier (e.g., Kossacki and Markiewicz, 2002; Titus *et al.*, 2006; Haberle *et al.*, 2008; Searls *et al.*, 2010). Vincendon *et al.* (2010) examined OMEGA (Observatoire pour la Minéralogie, l'Eau, les Glaces et l'Activité - Infrared Mineralogical Mapping Spectrometer) and CRISM data for the seasonal occurrence of CO<sub>2</sub> frost on steep pole-facing slopes in the southern hemisphere and found the timing of frost to be consistent with ground ice in the top meter of the surface layer. They concluded that shallow ground ice occurs on pole-facing 20°-30° slopes as far equatorward as 25°S, consistent with prediction of stable ground ice on poleward slopes by Aharonson and Schorghofer (2006).

Overall, the depth and geographic distribution of ground ice inferred from recent observations are consistent with previous findings (Beaty *et al.*, 2006), and

with vapor-diffusive equilibrium with atmospheric water as the primary controlling process. The occurrence of excess ice (ice greatly exceeding the pore volume of typical soils) in the subsurface and its variability on a wide range of length scales from < 1 m to >1000 km are, however, somewhat puzzling (see Mellon, 2012, for a discussion). While present day ice stability and its distribution appear to be controlled by diffusive equilibrium, the origin of the excess ice may involve other processes and potentially a role for liquid water or liquid-like thin films. These processes range from exogenic sources such as ancient flooding or dust-covered snowpack, to endogenic processes such as ice segregation and vapor deposition (Prettyman *et al.*, 2004; Feldman *et al.*, 2008; Mellon *et al.*, 2009b; Mellon, 2012; Sizemore *et al.*, 2014), which may operate during recent periods of higher obliquity or even in the current climate. Furthermore, since geologically-recent orbitally-driven climate change is expected to periodically desiccate and repopulate the upper meter or more of the permafrost (Mellon and Jakosky, 1995; Chamberlain and Boynton, 2007), emplacement of the concentrated ice would need to have occurred recently, since the last period of low obliquity (Mellon, 2012).

[Finding 5-7 Here]

[Figure 40 about here]

## **5.7 Radar detection of non-polar ice**

Both SHARAD on MRO and MARSIS on MEX have been used extensively for studies of ice on Mars. Radar sounding detects changes in the dielectric properties of materials, and is particularly sensitive to a subhorizontal interface between ice and rock (Gudmandsen, 1971). The polar deposits are generally more amenable to radar sounding due to their size, thickness, and nearly pure water ice content (Picardi *et al.*, 2005; Plaut *et al.*, 2007; Phillips *et al.*, 2008; Grima *et al.*, 2009); however, many features at lower latitudes have also been studied, revealing evidence for massive subsurface ice in multiple locations (Fig. 40), in a variety of forms.

Approximate vertical resolution is 10 m for SHARAD (Seu *et al.*, 2007) and 150 m for MARSIS (Picardi *et al.*, 2005), which trades resolution for deeper penetration. Horizontal resolution depends on orbital geometry, wavelength, and surface roughness, but in general the smallest features discernable with SHARAD are ~10 km across. Positive ice detection typically requires a deposit thicker than a few tens of meters, lying within a few tens of meters below the surface, and some means for constraining the dielectric constant, such as geometric constraints and/or attenuation. Correlation with surface morphology can bolster the identification of ice.

[Figure 41 about here.]

The most extensive mid-latitude ice deposits detected to date with radar sounding are viscous flow features (VFF; Holt *et al.*, 2008; Plaut *et al.*, 2009).

These typically surround massifs or abut escarpments in both hemispheres. Deuteronilus Mensae (Fig. 41) contains the most extensive and voluminous mid-latitude ice, where SHARAD shows that VFF covering large regions and filling valleys contain ice hundreds of meters thick (Plaut *et al.*, 2009a; 2010). A region lying east of the Hellas impact basin (Fig. 41) contains hundreds of DCG and some have been shown with SHARAD to be ice deposits over 700 m thick (Holt *et al.*, 2008). All DCG observed by SHARAD exhibit a single, discrete surface echo, implying that the thickness of the protective debris/dust cover is on order of the SHARAD vertical resolution (~ 10m or less). A large number of VFF, DCG, and glacier-like forms in general (Souness *et al.*, 2012) fall below the resolution threshold of SHARAD or lie in such topographically rough areas that subsurface echoes may be fully masked by surface “clutter” (Holt *et al.*, 2006).

Sheet-like deposits of ice-rich material (likely < 10% lithic content) spanning many thousands of km<sup>2</sup> have been detected in Arcadia Planitia (Plaut *et al.*, 2009b; Bramson *et al.*, 2014), Utopia Planitia (Nunes *et al.*, 2010; Stuurman *et al.*, 2014), and in Vastitas Borealis at the PHX landing site (Putzig *et al.*, 2014; Fig. 41), lying just below relatively flat surfaces and exhibiting thicknesses up to ~ 100 m. SHARAD coverage in the mid-latitudes is still rather sparse compared to the polar deposits, so the detection and mapping of ice there is an ongoing process.

The unit surrounding the south polar plateau known as the Hesperian Dorsa Argentea Formation contains a reflective horizon in MARSIS data over much of its mapped occurrence (Plaut *et al.*, 2007). The reflectors are observed at time delays consistent with a maximum depth between 500 and 1000 m. The relatively strong returns and the morphology of surface features both suggest an ice-rich layer overlying a lithic substrate. This implies the presence of a substantial additional H<sub>2</sub>O reservoir, consisting of ice that may be the oldest yet detected on Mars. In nearby Malea Planum, and in several mid-northern latitude locations, detections suggestive of ice have been made by SHARAD beneath ejecta blankets of a class of impacts known as pedestal craters (Nunes *et al.*, 2011).

[Finding 5-8 Here]

[Figure 42 About Here]

## **5.8 Spacecraft-induced deliquescence**

### *5.8.1 The Phoenix scoop*

In 2008 the PHX mission landed on a region of Mars nearer the North Pole than any earlier mission (68.22° N. latitude) in search of near-surface ice deposits. Ample evidence of ice was found by using first the landing thrusters and later the robot arm (Fig. 42) moving away surface material to reveal both clean and soil-laden ice at adjacent locations and just beneath thin layers of soil.

[Figure 43 About Here]

The mechanical behavior of soils was not always as predicted, resulting in difficulties delivering samples from the scoop to some instruments soil inlet ports. As seen in Fig. 43, the appearance and configuration of soil in the scoop changed over a period of time with no purposeful mechanical agitation. The difficulty in delivery has been ascribed to a “stickiness” property of freshly-acquired soil, which apparently diminished over a period of time upon exposure to the atmosphere. This viscid behavior has been ascribed to the possibility of deliquescence, especially with the discovery by PHX that martian soil contains perchlorate salts, whose low temperature properties are favorable to deliquescence when the martian relative humidity is high at nighttime. Exposure during the higher temperatures and lower relative humidity of daytime resulting in sublimation that reduced the water content below that required to maintain the deliquescent state.

[Figure 44 goes about here.]

### *5.8.2 The Phoenix strut*

During landing, the twelve descent engines (hydrazine monopropellant) were pulsed on and off to maintain a horizontal attitude and prescribed descent rate. The exhaust from these engines removed a layer of soil and exposed a flat surface of an apparent ice layer, as seen in the upper image of Fig. 44. One of the lander’s titanium struts could be imaged by the robot arm camera and was monitored during the mission because of blobs of material adhering to it and

exhibiting rounded shapes. These blobs, some of which showed changes during the course of the mission, have been interpreted as caused by possible deliquescent salts (Renno *et al.*, 2009). Although the relative humidity at the PHX site was at or near 100% during the coldest part of night, it was <5% during the daytime, and hence there was never an overlap in temperature and RH conditions with the zone of terrestrial habitability.

[Finding 5-9 about here]

## **6. The Implications and Opportunities of Special Regions Identification for Human Mars Missions**

Strong interest exists among various countries and private industries to send humans to Mars, both for short-term exploration and long-term colonization. Human activities on Mars would require access to life-sustaining resources, including water, oxygen, and protection from radiation, as well as the materials needed to create fuels for surface and launch vehicles. These resources would be available on Mars and would require access to surface or near-subsurface materials, some of which may be found in Special Regions. In particular, Special Regions are in part defined by the availability of water, making them a potential source of usable water and oxygen in addition to their science value. Protocols need to be established so that human activities do not inadvertently affect areas designated as Special Regions or cause non-Special Regions to become Special.

The spread of terrestrial biological contamination could impact life support systems as well as the availability of Mars resources to human explorers.

[Figure 45 goes about here.]

## **6.1 Availability of resources**

H<sub>2</sub>O in either liquid (water) or solid (ice) form would be the most important resource for human activities on Mars because it would be needed for human, plant, and animal consumption as well as for production of oxygen and many fuels (Beatty *et al.*, 2012a). It also can provide shielding from cosmic radiation that penetrates to the martian surface. The accessibility of H<sub>2</sub>O resources and whether these regions would be designated as Special depends on location (Fig. 45). The various resources that Mars provides regarding water, oxygen, radiation shielding, and fuel/power would be described in the following subsections and summarized in Table 10.

[Table 10 About Here]

### *6.1.1 Water resources*

The polar caps (between ~80° and 90° latitude in each hemisphere) would be the major reservoir of H<sub>2</sub>O that can be accessed by human explorers and would be not considered to be Special Regions. The seasonal caps covering these regions between autumn and spring are composed of thick deposits of carbon dioxide ice, but the permanent caps exposed during the summer are primarily H<sub>2</sub>O ice. Rheological and spectroscopic analysis of the permanent south polar H<sub>2</sub>O cap

indicate that it is covered by an ~8 m thick veneer of CO<sub>2</sub> ice even at the height of summer (Nye *et al.*, 2000; Titus *et al.*, 2003; Bibring *et al.*, 2004), which limits access to the underlying ice reservoir. However, the north permanent cap is estimated to be 90-100 wt% H<sub>2</sub>O ice, mixed with small amounts of dust from global dust storms, and is accessible at the surface. The cap is about 3 km thick and 1100 km in diameter. Its volume is estimated between  $1.1 \times 10^6 \text{ km}^3$  and  $2.3 \times 10^6 \text{ km}^3$  (Zuber *et al.*, 1998; Smith *et al.*, 2001). The freshwater content of the cap is estimated to be approximately 100 times the amount in the North American Great Lakes. However, polar night darkness, very cold temperatures, and the overlying CO<sub>2</sub> seasonal cap limit the period of time during which the H<sub>2</sub>O can be accessed. In addition, CO<sub>2</sub> degassing in the area, particularly in the spring, may negatively affect safe access by human explorers.

The region of Mars between 60° and 80° latitude in each hemisphere is largely covered by the seasonal CO<sub>2</sub> caps during the winter. As the seasonal caps retreat in the spring, frost outliers composed of both CO<sub>2</sub> and H<sub>2</sub>O ice are left behind, often within topographic depressions such as impact craters (Kieffer *et al.*, 2000; Armstrong *et al.*, 2005; 2007; Titus, 2005; Conway *et al.*, 2012). The region surrounding the north polar cap largely comprises the Vastitas Borealis Formation which is interpreted as being composed of ice-rich fine-grained (dust) deposits and ice-rich sediments from ancient fluvial activity (Tanaka *et al.*, 2008). Similar ice-rich fine-grained deposits are seen surrounding the south polar cap, but they

are much thinner than their counterparts in the north. Geomorphic features within this latitude range suggest ice-rich flow associated with glacial activity from past epochs as well as today (Kreslavsky and Head, 2002; Souness *et al.*, 2012). New fresh impacts in this region (Section 5.3) expose ice excavated from depths ranging from centimeters to a few meters (Byrne *et al.*, 2009b; Dundas *et al.*, 2014a). This latitude zone is not considered to be Special unless heated to the point where the ice melts. The accessibility limits of this region are the same as for the polar caps.

The mid-latitude regions (30°-60° latitude zone) retain geomorphic evidence of ice-related features that were emplaced during periods of high axial tilt (million year time scales) (Mustard *et al.*, 2001; Dickson *et al.*, 2012; Souness *et al.*, 2012, Sinha and Murty, 2013; Hartmann *et al.*, 2014). The region also retains geomorphic evidence of features produced by possible fluvial activity in the recent to distant past, such as gullies (Section 4.2) (Malin and Edgett, 2000a; Christensen, 2003; Malin *et al.*, 2006; Williams *et al.*, 2009; Johnsson *et al.*, 2014) and layered deposits on crater floors (e.g., Cabrol and Grin, 1999; Malin and Edgett, 2000b; Goudge *et al.*, 2012). Recurring Slope Lineae (Section 4.1) activity is concentrated in this zone, particularly in the southern hemisphere (McEwen *et al.*, 2011; Ojha *et al.*, 2014; Stillman *et al.*, 2014). This region also retains ice within centimeters to a few meters depth, as revealed though ice exposed by new small impact craters (Byrne *et al.*, 2009b; Dundas *et al.*, 2014a).

Ice deposition down to these latitudes occurs during periods of climate change associated with larger axial tilts (Head *et al.*, 2003). Although ice is plentiful in the near-surface within this latitude zone, this area is not considered to be Special except for the RSL sites. However, the ice-rich regions could become Special if heated to melting, or if some future observation points to the natural presence of water. Accessibility to the ice in this region is limited to the summer season if power is supplied by solar energy.

The equatorial region of Mars (between 30°S and 30°N) has limited locations of easily accessible H<sub>2</sub>O resources. RSL sites and potentially active gullies suggest the presence of near-surface liquid in certain locations and constitute Special Regions within this latitude zone. Areas of H<sub>2</sub>O enhancement identified from ODY neutron analysis within the equatorial region are usually interpreted as being due to hydrated minerals, which may contain water contents up to ~13% (Feldman *et al.*, 2004, 2008; Fialips *et al.*, 2005). Ice deposits from past periods of high axial tilt remain at depths >15 m in localized regions, such as northwest of the Tharsis volcanoes (Fastook *et al.*, 2008; Madeleine *et al.*, 2009). Impact crater analysis, radar data, and neutron spectrometer data suggest that subsurface ice is generally located at depths >5 m in this region and often at depths >50 m (Picardi *et al.*, 2005; Barlow *et al.*, 2007; Farrell *et al.*, 2009). Therefore, other than the RSL sites and possibly the active gullies, no location within the equatorial zone is considered Special. This region would be conducive to human activities due to the

high levels of solar energy and the warmest temperatures on the planet, but provides very limited access to H<sub>2</sub>O resources.

### 6.1.2 Oxygen

The martian atmosphere is composed largely of CO<sub>2</sub>, necessitating the production of oxygen through *In-Situ* Resource Utilization (ISRU) techniques to support human operations on the planet. This oxygen can be obtained either from the CO<sub>2</sub> and H<sub>2</sub>O in the atmosphere or the H<sub>2</sub>O resources in the planet's near-surface deposits. The amount of water vapor in the atmosphere varies seasonally but overall is a small amount compared to surface resources. Condensation of all H<sub>2</sub>O vapor in the atmosphere would produce a global layer with a volume of only ~1 km<sup>3</sup> of liquid (Barlow, 2008). Atmospheric CO<sub>2</sub> could be processed to provide the needed oxygen (Mars 2020 Science Definition Team, 2013). CO<sub>2</sub> electrolysis systems and water vapor condensers have high energy demands, which likely would require reliance on a nuclear reactor (MEPAG, 2010). In addition, dust in the martian atmosphere, particularly during dust storm periods, could clog atmosphere ISRU facilities.

[Figure 45 About Here]

Oxygen could be extracted from H<sub>2</sub>O deposits on the martian surface or near-subsurface (<3 m depth). Hydrated minerals, including phyllosilicates, sulfates, and carbonates, have been detected from orbiting spacecraft in localized regions

of the planet (Fig. 45) (Bibring and Langevin, 2008; Ehlmann and Edwards, 2014) and could be used to extract H<sub>2</sub>O and O<sub>2</sub>.

Perchlorate (ClO<sub>4</sub><sup>-</sup>) has been detected at the PHX and MSL landing sites (Hecht *et al.*, 2009; Glavin *et al.*, 2013) and in one martian meteorite (Kounaves *et al.*, 2014a). It is expected to be common in the martian regolith across the planet due to the mixing of fine-grained surface materials by dust storm activity. Davila *et al.* (2013) has suggested that perchlorate could be a source of ISRU-derived O<sub>2</sub> as well as propellants for surface and launch vehicles. However, perchlorate is known to impair thyroid function and therefore is toxic to humans. The presence of perchlorate in martian dust, groundwater, and in crops grown in martian soil would need to be reduced for human activities to be successfully conducted on Mars.

### *6.1.3 Fuel and power sources*

Fuel for surface operations and/or propellants for crew ascent to orbit could be manufactured from martian surface materials. Hydrogen, oxygen, and methane could be produced from atmospheric CO<sub>2</sub> or atmospheric/ surface H<sub>2</sub>O through electrolysis and the Sabatier process. The perchlorate found throughout the martian regolith also could be used to produce oxygen (Davila *et al.*, 2013). Martian surface materials contain various metals, including magnesium and aluminum, which could be mined for use as propellants (Ismail *et al.*, 2012).

Power for daily operations would be expected to be produced from solar energy and/or RTGs. As noted in Section 6.1.1, reliance on solar energy would limit year-round operations to the equatorial zone of Mars where near-surface/surficial H<sub>2</sub>O resources would be limited. Power from RTGs would allow surface operations at a range of latitudes, but heat produced from this source could result in some currently non-Special Regions becoming Special. For example, waste heat from RTGs powering a station located poleward of 30° latitude in either hemisphere could melt near-surface ice, resulting in liquid water ponds which could then become designated as Special Regions.

## **6.2 Radiation environment**

The thin martian atmosphere, small concentrations of atmospheric ozone, and lack of a present-day active magnetic field result in radiation reaching the martian surface from space. The RAD instrument on the MSL's Curiosity rover has measured GCR and SEP doses at the planet's surface and finds a GCR equivalent dose rate of 232 millisieverts (mSv) per year (Hassler *et al.*, 2014). The current federal occupational limit of radiation exposure per year for an adult is below ~0.05 Sv. A nominal 860 day human mission to Mars, with 360-day round-trip transit (180 days each way) and 500 days on the surface, is estimated to result in a total mission dose equivalent of ~1.01 Sv, based on MSL cruise and surface radiation measurements. Therefore shielding would be required for long-term surface operations on Mars. Deposition of regolith over surface habitats, water

storage (both in tanks and as ice) around habitats, or erection of habitats in underground environments such as lava tubes and caves would provide the necessary shielding from radiation to allow extended human activities to occur on the planet's surface, although caves and lava tubes may be Special Regions (Section 4.8). A few areas within the martian highlands retain crustal remnant magnetization from the early period when the planet possessed a magnetic field (Acuña *et al.*, 1999; Connerney *et al.*, 2004). This remnant magnetization may provide some partial shielding from cosmic radiation if human activities would be localized within these regions.

### **6.3 Limiting contamination of Special Regions by human activity**

Our group recognizes that it would not be possible for all human-associated processes and mission operations to be conducted within entirely closed systems while on the martian surface. The goal of human missions to Mars should be to not affect or otherwise contaminate Special Regions, nor be contaminated by materials from them (Race *et al.*, 2008). Human activities on the planet's surface therefore should take steps to avoid converting areas into Special Regions, such as through the melting of surface/near-surface ice by waste heat. This leads to the question: How can humans explore Mars in the desired level of detail while limiting contamination of Special Regions? One scenario would be to establish "safe zones" for human activities near Special Regions but only allow controlled robotic access to the Special Regions locations. This scenario implies that a

“clean” robotic rover can be landed in the same area as the human landing site and that the capability exists for humans to aseptically interact with the rover and receive contained, rover-collected samples. We can expect that other scenarios to avoid contamination with Special Regions would be advanced as human exploration of Mars comes closer to reality and current knowledge gaps would be removed by future discoveries and research (Beaty *et al.*, 2012b).

## **7. Discussion**

This study has been focused on the ability (or inability) of Earth organisms carried by spacecraft to replicate (and presumably keep on replicating) on or under the surface of Mars as we can envision it—or as we find it—sometime in the next 500 years (Finding 1-1). If this study were dealing with a complete data set, and we actually knew the capabilities of every Earth organism as well as each and every environment that is or will be on or under the surface of Mars during this time, we would still have unknowns and uncertainties associated with the “right” organism coming into contact with the “right” environment, and how that might chance to happen. Rest assured, however—we do *not* have a complete data set for either Earth life or for Mars environments. Thus, the unknowns and uncertainties associated with the identification of Special Regions on Mars will include a healthy dose of ignorance with respect to both.

Nonetheless, we have learned quite a bit about Mars since the previous MEPAG study (Beaty *et al.*, 2006), more about Earth life than we knew at that

time, too, and even a bit more about the spacecraft that will take (some) Earth organisms to Mars and their potential to create Special Regions on their own. Thus we are able to provide an update to the conclusions of that earlier study, even though our data sets remain woefully incomplete.

### **7.1 Environmental parameters used to define Mars Special Regions**

One of the assumptions built into this study and its predecessor is that the capabilities and limitations of the Earth organisms that may be carried *inadvertently* by spacecraft will be used to define the characteristics of *possible* Special Regions on Mars. While we see (Finding 2-1) that Mars is not easily shown to be lacking materials that could support some Earth organisms (e.g., chemolithoautotrophs), we also do not place limits on the ability of any Earth organism small enough to do so to stow away to Mars (Findings 2-2, 2-3). There may be such limits, but our ignorance of the microbial world, and the variety of transport processes that could result in a microbe boarding a spacecraft, are sufficient to make their imposition impractical. And we cannot limit our stow-aways to chemolithoautotrophs. It is possible that even organisms that depend on metabolizing organic compounds could be accommodated on Mars, somewhere (Finding 2-4)—or maybe everywhere, badly, given the small amounts of organics so far detected. In order to take a conservative approach to the identification of potential Special Regions on Mars, as did the 2006 study, we start with the most

basic characteristics of an environment—ones that can be shown to affect *all* microbes on Earth.

*7.1.1 Recommended organism-based parameters defining the limits of life, and the requirements for Mars Special Regions: T and a<sub>w</sub>*

Conditions on the surface of Mars are often described as being “cold” and “dry” (along with dusty and cratered). As it happens, those conditions are critical to the ability of Earth organisms to replicate in any environment. If it is too cold (or too hot), or too dry, Earth microbes will not replicate. Thus we define the basic parameters of a Special Region (without margin) as a location where:

- (1) the temperature (T) is 255K (-18°C) or above (Finding 3-1), and
- (2) water activity (a<sub>w</sub>) is above 0.60 (Finding 3-4).

While it can be shown that organisms can be more sensitive to a<sub>w</sub> than the accuracy of measurement suggested by a value of 0.60, the practicality of measuring water activity at the same accuracy as an organism senses it has not yet been established.

Under the definition adopted by MEPAG in 2006, “if a martian environment can *simultaneously* exceed the threshold conditions the threshold conditions of -20°C and a<sub>w</sub> over 0.5, propagation may be possible” (italics added). Both of those parameters in the 2006 had margin placed on them, both to lower the temperature as well as the water activity required for describing a location as an Uncertain region, which could be expected to host microbial life if it were introduced

therein. *With equivalent margin* added, the basic parameters of a Special Region would describe a location where:

(1) the temperature (T) is 250K (-23°C) or above, and

(2) water activity ( $a_w$ ) is above 0.50.

#### *7.1.2 Organism-based parameters not at the limits of life, and thus not defining Special Regions*

A number of other organism-related parameters were considered with respect to the martian environment, and found not to be close enough to the limits of life, or to allow us to map those limits well enough to be used to define Special Regions. For example, compounds known as chaotropes can lower the temperature at which an organism can replication (Finding 3-3), but there is no record of chaotropes enabling replication at temperatures below 255K (-18°C) and the variety of salts that can act as chaotropic compounds are not localized on the martian surface. Other parameters may not be useful as discriminators because the physical conditions on Mars (outside of T and  $a_w$ ) are not sufficiently challenging to eliminate the possibility of Earth-life living there. For example, low total pressure (below 2,500 Pa) does not prohibit some Earth organisms from replication (e.g., at 700 pascals, where water will remain a liquid at temperatures at 0°C or slightly above; Finding 3-6), and the Mars UV environment, while generally lethal to Earth microbes, can be shielded easily by dust or by other organisms (Finding 3-7). Measurements of radiation due to GCR and SPE are

more benign than previously anticipated (Finding 3-8), and are fairly uniform with respect to location on the martian surface (although being buried alive is a useful way to avoid this radiation altogether). Making the entire set of issues more complex is that some microbes (and especially mixed communities of microbes) are more likely to survive multiple, differing stressors than they are to survive those stressors when faced with them one at a time (Finding 3-9). Whatever the confounding issues, the SR-SAG2 (like its predecessor) has found that Mars Special Regions should only be defined by measures of temperature *and/or* water activity.

#### *7.1.3 A non-equilibrium Mars and asynchronous conditions related to life*

While humanity's efforts in Mars exploration have continued to expand since the era of the Mariners 4, 6, 9, and the Viking missions, we are still challenged in our ability to take observations made at orbital distance (or farther) and translate them (with or without an intervening lander; see Table 6) into an understanding of a specific environment, over time, at a scale that is applicable to the survival, growth, or even replication of microbial life (Finding 3-10). As such, we are hindered by our size, the size of our spacecraft, and the size of Mars—as well as our perception of time. Microbes can live their lives much more quickly than we do, but also much more slowly. And even on Earth we are only now beginning to appreciate the contributions and abilities of organisms that form over 50% of the

Earth's biomass (cf., Whitman *et al.*, 1998). How they will adapt to Mars environments we have yet to categorize is a puzzle that we would like to solve.

In the discussion above and in the earlier 2006 study it was implicit that Mars Special Regions must be defined by appropriately warm temperatures *and* enough water activity occurring together in the same place and in the same time. Were their intersection to have been mapped out on the martian surface, the 2006 study's expectation for warm temperatures and high-enough water activity (Beaty *et al.*, 2006, Fig. 8, p. 700) would have been a blank map, as the posited Mars subsurface equilibrium conditions did not allow warm-enough temperatures and sufficient water activity to coexist. As such, no natural Special Region would exist outside of (possibly) gully systems or other non-understood features, or in the deep subsurface. By specifying a water activity value, one was automatically faced with a temperature that was too low to allow Earth organisms to replicate, and if the temperature was high enough for that, the relative humidity at that site would be excruciatingly low, allowing no replication on its part.

In this study the specific examples cited in the 2006 report were affirmed, but more attention was paid to the regular, even cyclic, disequilibria in temperature and water activity demonstrated at the Viking 1, PHX (Thermal and Electric Conductivity Probe, TECP) and MSL (REMS) landing sites (Figs. 26, 27), where in some seasons the temperature required for microbial replication was regularly reached during the driest part of the day, whereas at night, when the temperature

was too low for replication, the relative humidity at the site was above 0.6 and nearly always close to 1.0. The non-overlap of the required values for a Special Region is reflected in Finding 4-12, but the fact that both could be reached within the same 24 hour period, regularly, suggests that there may be a way for organisms to connect the favorable aspects of those periods across a bridge of biotic adaptation.

At present, we do not have any evidence that Earth organisms can build that bridge. While Finding 3-11 encourages a future rigorous look at the specific capabilities of the Earth's lichens in Mars conditions, and Finding 3-12 provides some circumstantial evidence relative to the question, there is much work to be undertaken to show that any Earth organism can live under the changing conditions seen by the landers of Fig. 27. In fact, Finding 3-13 suggests that those conditions may be unbridgeable, with low water activity matrix effects in the shallow subsurface dominating microbial survival, let alone reproduction.

Other non-equilibria may also occur on Mars, and in understanding those we are hampered by a lack of observations and experience. For example, the Whiteway *et al.* (2009) reported observations from the PHX LIDAR, included some measurements of that precipitation reaching the ground, generally in the early morning hours. Finding 3-14 reminds us that it is at least theoretically possible that in the parts of Mars where the total atmospheric pressure is above the triple-point of water, that precipitation could be subjected to transient melting

(and it could be aided in that melting if it fell on a salty surface). How important would that be locally? Without having seen it occurring and being able to measure the related phenomena (including the mineralogical effects) it is simply impossible to tell. We need more experience with those parts of Mars where it *could* occur, and we need to be able to make related observations in the dark.

Other mechanisms might also lead to narrowing the gap between high enough temperatures and sufficient water activity to make something interesting happen biologically. Finding 3-15 deals with the expectation that certain materials, whether in the local environment (e.g., clays) or as part of the organisms themselves (e.g., certain proteins) can allow microbes to retain water more capably than a shallow-subsurface equilibrium model built on average soil properties would predict. The understanding of these phenomena at the microbial scale represents a potentially productive contribution to our understanding of Mars Special Regions in the future.

## **7.2 Environments on Mars: a proposed categorization**

At our current stage of observational familiarity with the martian surface and subsurface (as far as SHARAD and MARSIS can see, ~1 to 1.8 km, respectively), our understanding of the processes that have shaped the planet is far from complete. Hence, something that looks like a gully found on Earth is called a “gully” although there may be numerous reasons for that particular landform to be in that particular place, with similar-looking landforms being shaped by different

processes. Likewise, a single process, when faced with a multiplicity of different landforms may shape each of them differently. As such, there are different implications for the identification of Special Regions in very similar looking parts of Mars.

*7.2.1 Parameters considered in categorizing natural environments, but not used*

Table 7 summarizes the potential microscale environments anticipated on Mars, and the SR-SAG2 evaluated their likely contribution to the existence of naturally occurring Special Regions, accordingly. As a result of this evaluation, the Group did not define any Special or Uncertain Regions on the basis of vapor-phase water availability (see above), ice or brine-related sites (exclusive of temperature and water availability criteria that govern both ice-related phenomena and the deliquescence of salts), or aqueous films or water in minerals (finding the water bound too tightly to be of use to microbes, based on the water activity criterion). See Findings 4-13, 4-14, and 4-15. The potential for periodic condensation or dew to form (along with the frost first observed at the Viking 2 site) was noted (Finding 4-16), but there is not enough data to ascribe possible Special Regions to those phenomena.

*7.2.2 Parameters used in categorizing natural environments*

Again referring to Table 7, the remaining micro- or macroenvironments of relevance to Special Regions are groundwater and possible thermal springs on

Mars. Neither of these have been observed on or under the surface of present-day Mars, but there is ample evidence to suggest their existence on the relatively recent Mars ( $\leq 10$  mya). As such, their effects on landforms on the surface of Mars are the determinants of environments that may be Special Regions, and in this evaluation will be designated as Uncertain Regions—to be treated as Special Regions.

[Table 11 here]

### **7.3 Natural Special Regions / Uncertain Regions: classification and guidelines**

Table 11 contains the proposed classification of features comprising Special and Uncertain Regions on Mars (as well as those now thought to be Non-Special). The classification of RSL, best explained by the seepage of water at  $>250$  K, with an unknown, and perhaps variable  $a_w$ , reflecting Finding 4-1. The classification of gullies from Findings 4-2 and 4-3, as well as Table 9 is reflected here. On the conservative side, observed gullies whose formation and activity is inconsistent with liquid water, but consistent with  $\text{CO}_2$  as the active fluid, are considered Non-Special, but the rest are considered as Uncertain Regions.

That classification is justified, given that most of the current gully activity on Mars for which seasonal constraints are available (by means of careful change detection surveys by the HiRISE instrument on MRO) occurs at the  $\text{CO}_2$  frost point, and is thermally incompatible with the presence of liquid water. Rare

activity seen at warmer temperatures is consistent with dry mass wasting on steep slopes.

Some gullies show erosion that may have been accomplished by liquid water, most likely in a prior (warmer) climatic environment. If so, such liquid could have originated through the melting of surficial ice deposits that had been laid down in the last glacial period, which culminated a few hundred thousand years ago. Nonetheless, there is nothing in either the MARSIS or SHARAD data sets that is suggestive of shallow groundwater origin for any of the gullies (any associated reservoir of subsurface liquid water should be clearly visible in the orbital radar data). Thus, the potential for a gully to have liquid water during the next 500 years is primarily dependent on (1) its association with residual ice that has not yet melted, or (2) its association with RSL, for which a water-related genesis is possible but not proven. That potential is considered carefully in the gully classification scheme shown herein.

Table 11 also includes features that would be considered to be Special Regions if they were observed, but have not yet been seen. These include recent craters that are still warm (ref., Finding 4-4), groundwater (ref., Findings 4-5, 4-6, 4-7) and thermal zones (ref., Finding 4-10). Table 11 also reaffirms conclusions reached by the 2006 study (Beaty *et al.*, 2006), with Findings 4-8 and 4-9 leaving Polar dark dune streaks and slope streaks (that are not RSL) in the Non-Special classification.

Finally, Table 11 also includes martian caves in the Uncertain Region classification. As reported in Finding 4-11, the extent of these geomorphic features on Mars is currently not known (but see Fig. 24), though the potential for them to provide significantly different environmental characteristics from the surface is significant.

It should also be noted that Table 11 does not address spacecraft-induced Special Regions (see Finding 5-1), which are discussed below.

### *7.3.1 Map products*

Following the definitions for natural and spacecraft-induced Special Regions on Mars (see Sections 4 and 5), five map units are specified that meet criteria for potential spacecraft-induced or natural Special Regions. The units are defined on the basis of spacecraft observations and theoretical considerations for potential surface and near-surface transient water and residual water ice (Fig. 46). The map unit boundaries in some cases have large spatial uncertainties.

### *7.3.2 Unit 1: Continuous shallow ice within 0.3 m of the surface.*

This unit is based mainly on a theoretical model by Mellon *et al.* (2004, Fig. 9b) that has been validated by spacecraft observations (see also Section 4.9). Sections bounded by dotted line segments show where the 6 count-per-second epithermal neutron boundary occurs equatorward of the model boundary as determined by Mellon *et al.* (2004); in these areas the unit boundary is highly

uncertainty. Otherwise, boundary location uncertainty is on order of 100 km, which is the approximate width of the red lines in Fig. 46.

As suggested by Finding 5-6, this unit may also show indications of shallow ice in the form of polygonal ground. The exact relationship of this Unit to the formation of excess ice (Finding 5-7) in the shallow subsurface of Mars will be of import in later assessments of the likelihood of Spacecraft-Induced Special Regions as a consequence of landings in this Unit.

Unit 1 also encompasses unmapped detections and inferences of local, generally >1-5 m deep ice as indicated by: (a) Recent impact craters exposing ice within 1-2 m of the surface (Dundas et al., 2014a) (see also Section 5.4 and Finding 5-5); (b) geomorphic features interpreted to be ice-cored glaciers (some of which include SHARAD detections consistent with buried ice at depths of tens of meters; see Sections 5.3 and 5.7; Finding 5-8) (Dickson *et al.*, 2008; Holt *et al.*, 2008; Plaut *et al.*, 2009); (c) SHARAD detections consistent with buried ice at 20-50 m depth in planar materials in Arcadia Planitia (Bramson *et al.*, 2014) (see also Section 5.7); and (d) widespread locations of unmodified to partly deflated, ubiquitous mid-latitude mantle material (Mustard *et al.*, 2001) See Finding 5-4.

### *7.3.3 Unit 2: Discontinuous shallow ice within 5 m of the surface.*

This unit follows the 1 m local slope stable ice boundary of Aharonson and Schorghofer (2006; Fig. 9f) (see also Section 5.6). The boundary location uncertainty generally is on order of 100 km, which is the approximate width of

the blue lines in Fig. 46. Unit 2 also encompasses unmapped detections and inferences of local, generally >5 m deep ice as described in association with Unit 1. See Finding 5-3.

*7.3.4 Unit 3: Shallow ice absent, potential for ice >5 m deep.*

Ribbed deposits occurring on western flanks of Tharsis shields are interpreted to be largely desiccated TMG (Head *et al.*, 2003) (see also section 5.2; Finding 5-2). These deposits coincide with the Late Amazonian apron unit (IAa) mapped by Tanaka *et al.* (2014), which is the mapping shown here.

[Figure 46 About Here]

*7.3.5 Unit 4: Possibility of transient surface water inferred from RSL.*

RSL are recently formed, dark slope features identified in HiRISE images over multiple Mars years when surface  $T > 250\text{K}$ . These features include both confirmed and partly confirmed RSL as defined by McEwen *et al.* (2014) (see also Section 4.1). RSL typically occur in areas hundreds of meters to kilometers across. RSL locations are indicated as 50-km-diameter, circular Special Regions in order to provide for adequate precautions for spacecraft landings in their proximity, including an allowance for the possibility of an off-target landing (Fig 47).

[Figure 47 goes about here.]

*7.3.6 Unit 5: No shallow ice within 5 m of the surface observed or suspected.*

No evidence for or reason to suspect surficial or shallow ice or water exists in this region.

#### **7.4 Proposals for analyses to support future-mission planetary protection requirements**

##### *7.4.1 RSL, gullies, and (someday) caves*

High-spatial-resolution monitoring was required to detect the presence of RSL, and high-temporal-resolution monitoring was required to confirm the presence of RSL and distinguish them from other types of slope lineae that may look similar. Other features may have similar characteristics to RSL (perhaps involving water), but their characteristics are not identical. RSL may or may not be associated with gullies, as well. Only a limited number of caves have been identified, but it is anticipated that more will be identified in the future.

In order to prevent the inadvertent landing of a spacecraft near one of these features, in support of its overall planetary protection categorization, it is proposed that any mission whose landing ellipse or proposed area of operations will include RSL, one of the gully features designated as an Uncertain Region, or a cave, should prepare an analysis of the following:

- Any case that can be made to constrain the age of activity in the specific RSL or gully feature (active, fossil, or unknown), or the nature of the cave;
- Any constraints identified with respect to whether shallow groundwater is or is not present in the area of the feature;

- Whether/how the mission is intended to, or might, interact with the identified feature; and
- Consequences of various failure modes associated with the mission's EDL profile, and the expected landing location of each.

#### *7.4.2 Other spacecraft-induced Special Regions*

An accurate evaluation of the possibility of Special Regions induced by future spacecraft is highly dependent on the nature of those spacecraft, their heat sources, and their landing locations. Only general guidelines are thus possible at this point. Nonetheless, all surface missions will perturb the local thermal environment to some extent. For missions sent to a location underlain by ice, proposers should evaluate the possibility of:

- Melting to form liquid water/brine;
- The amount of time that liquid might exist;
- The location(s) to where it might migrate; and
- What its ranges of water activity and temperature could be.

For missions sent to a location not underlain by ice, proposers should evaluate the potential presence of highly hydrated salts which, upon heating, could form brine via deliquescence (Finding 5-9). Evaluate this via modeling the environmental conditions of temperature, water activity, and composition of the brine for comparison to the environmental limits for cell division for terrestrial organisms. In addition, evaluate the possibility of the brine as a transport

mechanism for terrestrial microbes to known or unknown subsurface environments.

Thermal perturbation of the local environment by a spacecraft could induce localized Special Regions, so the thermal environment induced onto the surface and near-surface regolith by the spacecraft should be analyzed for each landed mission. For spacecraft carrying one or more radioisotope heat sources, analyses should be performed to evaluate the probability, extent, and lifetime of each Special Region that could be created by both normal and anomalous events.

### **7.5 Knowledge Gaps**

There are major gaps in our understanding of life and Mars that, if filled, would add powerful insights into Mars astrobiology and clarify planetary protection issues associated with Special Regions. These are listed here without regard to possible priority:

- The synergy of multiple factors that enable enhanced microbial survival and growth (i.e., storage mechanisms; biofilms, and the structure of microbial communities), and mechanisms that may allow for temporal separation in microbial resource use.
- Studies that consider varying multiple extreme parameters, especially those that trade simplicity for robust generation of multifaceted stresses.
- Investigations into microbial activity at low water activity – additional physiological studies on the limits to microbial life.

- Investigations into microbial activity at lower temperature limits for life – additional physiological studies under controlled conditions with a mix of varied parameters (including temperature, water activity, chaotropic activity, etc.).
- Investigations into the properties of various minerals in harsh conditions, such as clays, zeolites and other three- dimensional minerals (for example, sulfides), that may affect their ability to support microbial life.
- Further research into excess ice, and mixtures of ice and salt at the PHX landing site.
- Extend our existing martian datasets in four areas:
  - Detailed change detection surveys by the HiRISE instrument and follow-ons, and research to understand contemporary processes driving RSL and gully activity.
  - Extend the coverage of the radar surveys by MARSIS and SHARAD.
  - Continued thermal mapping by THEMIS.
  - Continued observations from the ground at Gale Crater by REMS or elsewhere by REMS-like instruments.
- Further investigations into caves on Mars
  - Expand the survey to expand the number of known caves on Mars

- Investigate or model the behavior of frozen volatiles that may be trapped in Martian caves (cf., MacDonald, 1993; Ford, 2007; Williams *et al.*, 2010)
- Investigate the potential differences in atmospheric characteristics in caves on Mars versus the surface (cf., Hose *et al.*, 2000; Boston *et al.*, 1992). However, while this is possible on Mars, no evidence currently exists to assist us with this question. Future identification of any point sources of anomalous gases coming from the subsurface should be assessed for whether subsurface cavity or fracture habitat might exist at such a site.
- Understand the likely temperature profile in martian caves of different depths resulting from positive geothermal heat flow.

## **8. Summary**

In the light of new information and understanding about Mars environments and Earth microbes, we have revisited and revised the definition (and the interpreted locations) of Special Regions on Mars. A two-step process was used to update our understanding/interpretations of Mars Special Regions to include and examination of the literature for the limits of microbial life, the availability and action of water on Mars, and the specific features or depths in which habitats related to life might be found. We have updated our understanding of the environmental limits to microbial reproduction on Earth as well as the known

and/or hypothesized environmental conditions on Mars capable of sustaining them. In addition to planetary protection consequences, we have noted implications of this information to the presence and availability of related resources on Mars to support future human exploration.

Special Regions on Mars continue to be best-determined by locations where both of the parameters (without margins added) of temperature (above 255K) and water activity ( $a_w$ ;  $>0.60$ ) are attained. There are places/times on Mars where both of these parameters are attained within a single sol, but it is unknown whether Earth organisms can use resources in this discontinuous fashion. No regions have been definitively identified where these parameters are attained simultaneously, but a classification of landforms on Mars leads to RSL, certain types of gullies, and caves being named Uncertain Regions, which will be treated as if they were Special Regions until further data are gathered to properly classify them as Special Regions or Non-Special Regions.

Thus, during the planning phases, missions will study their own potential to create spacecraft-induced Special Regions by the presence of a lander, itself, or by non-nominal operations during the descent phase, and take action to ensure that Special Regions are not inadvertently created. Robotic spacecraft will need to avoid Special Regions if they are not clean enough to avoid contaminating those Regions. Although current requirements are the same as those met by the Viking

missions of the mid-1970s, no spacecraft sent to Mars since that time has been clean enough to enter a Special Region.

Human explorers require access to in-situ resources, some of which may be found in Special Regions. Water and oxygen for ISRU are found in the atmosphere, surface/near-surface ice, hydrated minerals, and perchlorates. Water ice is most abundant at latitudes poleward of ~60 degrees, but polar darkness, cold temperatures, and CO<sub>2</sub> degassing present hazards to human operations in these regions. Accessible water is more limited toward the equator, though temperature and solar energy conditions become more favorable. RSL may be liquid water of limited salinity, although they could be difficult to tap given their location on difficult slopes and the need to avoid contamination of them, and any aquifer that may be associated with them, if they are to be usable by human explorers or objects of further scientific study.

Fuel for surface operations and propellants for crew ascent could be manufactured from the martian atmosphere and surface materials, but dust in the atmosphere may clog ISRU equipment and perchlorate is potentially toxic to humans (thyroid effects) if it reaches higher concentrations in the habitat or suit atmosphere. Power may also be produced from solar or nuclear energy, although reliance on solar energy may limit operations to the equatorial zone of Mars, where easily accessible ice resources are limited. Nuclear power could allow surface operations at a range of latitudes, but care must be taken to prevent waste

heat from converting some Non-Special Regions into Special Regions. Radiation shielding is necessary for long-term human operations on Mars and could be obtained by deposition of regolith or by water storage around habitats, either in tanks or as ice. It will be impossible for all human-associated processes and operations to be conducted within entirely closed systems, so protocols need to be established so (1) human missions to Mars will not contaminate Special Regions nor be contaminated by materials (or possibly organisms) from them, and (2) human activities on Mars will avoid converting Non-Special Regions to Special Regions, and thus help control the spread of Earth microorganisms on Mars.

**Acknowledgements:**

The committee reported its analysis at the COSPAR Special Regions Workshop (April 1-3, 2014) held in Montreal and at the 29<sup>th</sup> MEPAG Meeting (May 13-14, 2014) held in Washington, D.C. The discussions that commenced were valuable in clarifying and ensuring completeness of the report. The following scientists provided data, data interpretation, consultation, or ideas to this analysis: Philip Ball (London), Phil Christensen (Arizona State University), Steve Clifford (Lunar and Planetary Institute), Jonathan A. Cray (Queen's University Belfast), Jay Dickson (Brown University), Colin Dundas (U.S. Geological Survey), Ailsa D. Hocking (Queen's University Belfast), Jack Holt (University of Texas Institute for Geophysics), Joe Levy (University of Texas Institute for Geophysics), Roger Phillips (Washington University), Jeff Plaut (Jet

Propulsion Laboratory/California Institute of Technology), Than Putzig (Southwest Research Institute), Andrew Stevenson (Queen's University Belfast), David J. Timson (Queen's University Belfast), and Tetsu Tokanaga (Lawrence Berkeley National Laboratory). The committee would like to recognize Trent Hare (USGS) for composing the Mars GIS map featured in this report. J. Hallsworth received funding from the Enterprise Directorate of Queen's University Belfast. Authors Dave Beaty and Melissa Jones were supported by the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

**Author Disclosure Statement:**

The authors declare no competing financial interests relative to this manuscript.

**Abbreviations/Acronyms:**

$a_w$ , water activity; CCF, concentric crater fill; COSPAR, Committee on Space Research; CRISM, Compact Reconnaissance Imaging Spectrometer for Mars; CTC, 5-cyano-2,3-ditolyl-tetrazolium chloride; DCG, debris-covered glacier; DNA, deoxyribonucleic acid; DRH, deliquescence relative humidity; DT, doubling time; EDL, entry, descent, and landing; ESA, European Space Agency; GCR, galactic cosmic ray; GIS, Geographic Information System; GPHS, general purpose heat source; HiRISE, High Resolution Science Experiment; ISRU, In-

Situ Resource Utilization; ISS, International Space Station; JPL, Jet Propulsion Laboratory; LD<sub>90</sub>, lethal dose; LDA, lobate debris apron; LIDAR, Light Detection and Ranging; LVF, lineated valley fill; MARSIS, Mars Advanced Radar for Subsurface and Ionosphere Sounding; MEPAG, Mars Exploration Program Analysis Group; MER, Mars Exploration Rovers; MEX, Mars Express; MGS, Mars Global Surveyor; MOC, Mars Orbiter Camera; MOLA, Mars Orbiter Laser Altimeter; MRO, Mars Reconnaissance Orbiter; MSL, Mars Science Laboratory; NASA National Aeronautics and Space Administration; ODY, Odyssey; PHX, Phoenix; PLD, polar layered deposits; RAC, Robotic Arm Camera; RAD, Radiation Assessment Detector; RAT, Rock Abrasion Tool; REMS, Rover Environmental Monitoring Station; RH, relative humidity; RMC, ring mold crater; RSL, recurring slope lineae; RTG, radioisotope thermoelectric generator; SEP, solar energetic particle; SHARAD, Shallow Radar; SPE, solar particle event; SR-SAG2, Special Regions Science Analysis Group 2; SSI, Stereo Surface Imager; T<sub>E</sub>, eutectic temperature; THEMIS, Thermal Emission Imaging System; TMG, tropical mountain glacier; UN, United Nations; UV, ultraviolet; VFF, viscous flow features.