Martian Extremophiles? – The H_2O_2-H_2O Hypothesis and its Implications for the Mars Phoenix Mission. D. Schulze-Makuch[1] and J. M. Houtkooper[2], [1]School of Earth and Environmental Sciences, Washington State University, Pullman, WA 99164, USA, dirksm@wsu.edu, [2]Center for Psychobiology and Behavioral Medicine, Justus-Liebig-University of Giessen, D-35394 Germany, joophoutkooper@gmail.com.

Introduction: The Mars Phoenix Lander Mission is set to launch in August 2007 for arrival on Mars in May 2008. The targeted landing site is in the subpolar northern region between 65 and 75 north latitude. We suggest that this region has a high potential to be populated by microbes and thus warrants bolder strategies to allow the possible detection of active microbial life in the shallow subsurface. The Martian surface is usually considered uninhabitable, but organisms could exist in the near-surface [1,2], especially if aided by special adaptation mechanisms such as the incorporation of hydrogen peroxide inside their intracellular fluids (referred to as H_2O_2-H_2O hypothesis [3]) which would aid organismic survival in the harsh Martian environment.

Environmental History of Mars: While the issue of stable liquid water on the Martian surface cannot be satisfactorily resolved at the present time, recent geomorphological, geochemical, and elemental evidence compiled from orbiting spacecraft (e.g., Mars Express and Mars Odyssey, and the MER rovers Opportunity and Spirit), seems to confirm a wet Mars during some time in its early recorded history [4-5]. A warm and wet global climate may have occurred at least into the Middle Noachian followed by persisting dry and cold periods interrupted mainly by short-duration pulses [6] of endogenic-driven activity, especially at Tharsis and the surrounding regions [7]. Catastrophic flooding and ponding occurred in the northern plains through time to form bodies of water ranging in size from oceans to lakes [8]. Thus, the emergence of microbial life in analogy with Earth seems reasonable and the northern plains may have been a prime location for microbial diversity and biomass during the Noachian. Life on Mars presumably would have spread from its point of origin to populate most of the planet, becoming abundant in liquid surface water pools and early oceans [9]. As the environmental conditions on Mars deteriorated, any microbial organism would have to adapt to the progressively more challenging conditions. Life, once evolved on Earth, proved to be extraordinarily adaptive through natural selection. Despite numerous global catastrophes and recurrent environmental changes, several of which extinguished a large proportion of the existing species, life has persisted to occupy every suitable habitat on the planet. Life on Mars could likewise be expected to have been fairly tenacious, adapting to the global changes that occurred as the planet passed through cycles of cooling and dessication [9].

There are three major options of life to adapt to Martian conditions: (1) to retreat into a nutrient-deprived subsurface environment adopting a psychrophilic life style, (2) employing a life style between dormant microbial states utilizing spore-formation or cryptobiotic options and a proliferate life-style during time periods with abundant liquid water on the Martian surface [9], or (3) to adapt via biochemical changes to the current environment on Mars, which is discussed below.

Biochemical Adaptations to the Changing Martian Environment: Many approaches to adapt to extreme environments are known from Earth including adaptation to some of the same stresses that occur on current Mars (e.g., cold temperatures, low pressure, lack of liquid water, and an intense radiation environment). However, the combination of stresses on Mars is quite unique and is not exhibited in any Earth environment. Thus, it is reasonable to expect that a novel adaptation mechanisms could evolve, one that is not exhibited on Earth. One radical solution would be the inclusion of hydrogen peroxide in the intracellular fluids. This would have the advantage of (1) lowering the freezing point significantly (the eutectic is at -56.5°C), (2) mixtures of high H_2O_2 concentrations supercool and don't form ice crystals (thus would not pierce cellular membranes upon freezing), (3) providing a source of oxygen, and (4) exhibition of hygroscopic properties thus allowing to scavenge water directly from the atmosphere [3,10]. Furthermore, both photosynthetic and chemosynthetic metabolic reactions would theoretically be feasible [3].

$$CO_2 + 3\ H_2O + \text{Sunlight} \rightarrow CH_2O + 2\ H_2O_2 \quad (1)$$

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \quad (2)$$

H_2O_2-H_2O solutions are mostly known as desinfectants and sterilizing agents on Earth. Thus, the compatibility of H_2O_2 with biological processes might seem questionable. However, some microbial organisms produce hydrogen peroxide (e.g., certain *Streptococcus* and *Lactobacillus sp*. [11,12], while other microbes utilize H_2O_2 (e.g., *Actinomyces viscosus*, and *Staphylococcus epidermidis* [12]). The microbe *Acetobacter peroxidans* even uses H_2O_2 in its metabolism (overall reac-

tion $H_2O_2(aq) + H_2(aq) \leftrightarrow 2H_2O$ [13]). Organisms control the high reactivity of H_2O_2 usually with the help of stabilizing compounds. H_2O_2 is also commonly used as a defense mechanisms by microbes, mammalian cells, and even certain insects (e.g., the Bombardier beetle uses a 25% solution of H_2O_2). Organisms also produce H_2O_2 to mediate diverse physiological responses such as cell proliferation, differentiation, and migration [14,15]. Thus, high concentrations of H_2O_2 can be produced and utilized biochemically even by Earth organisms.

Implications for the Mars Phoenix Mission: If organisms employ a H_2O_2-H_2O mixture as intracellular fluid, the northern plains in the higher latitudes would be a prime target to detect these putative organisms. In these latitudes conditions for the putative microbes would be relatively benign due to high water vapor concentrations in the atmosphere [16], temperatures not higher than 260 K [17], and evidence of geological activity (e.g., erosion). An abundance of liquid water would be harmful, and this might have been the reason why the Viking Life Detection experiments were unsuccessful to grow microbes (see Companion LPSC 2007 abstract "Detecting Life on Mars: Reanalysis of the Viking Life Detection Experiments and the Role of H_2O_2 as a Possible Biological Agent" by J.M. Houtkooper and D. Schulze-Makuch). The Viking experiments were conducted under too warm and wet conditions according to the H_2O_2-H_2O hypothesis for Martian life [3] exposing the putative organisms to extremely unmartian condition. Any organism tested would have been killed by hyperhydration and excessive heat.

The Microscopy, Electrochemistry, and Conductivity Analyzer (MECA) on the Mars Phoenix Lander would be an ideal instrument to probe for putative Martian organisms and is in many aspects superior to the Viking instruments. H_2O_2 and the release of dissolved O_2 upon wetting could be directly detected, which would support the case for possible H_2O_2-H_2O-based life on Mars. However, this would not be conclusive proof as it could be argued to occur non-biologically due to an unknown chemical H_2O_2 source or some unknown strong chemical oxidizer that might release H_2O_2 in a chemical reaction (as proposed as explanation for the Viking observations), and thus only provide circumstantial evidence. However, given the instrumentation suite on Phoenix a bolder approach could be entertained. Some of the 69 different substrates transported to Mars could be used to attempt growing organisms. The Viking experimental design to require metabolism is generally considered one of the major mistakes of the Viking Mission. However, this approach is not intrinsically flawed. Instead, the problem was that the experiments were designed and conducted when we knew too little about the environmental conditions on Mars. Thus, addressing the lessons from the Viking Mission, care should be taken not to overwhelm any organisms with too much water, heat, and nutrients. Instead, nutrients should be provided at low but variable concentrations (including some limited CO_2, H_2O, organics, N, P) and the microscopes could be used to monitor any changes. Ideally, microbial populations would grow to colonies to a size that could be imaged by the optical microscope on board the lander. The Phoenix Mission will provide a landing at a suitable place with suitable instrumentation to explore biota based on a H_2O_2-H_2O mixture as intracellular fluid.

Summary: The use of a hydrogen peroxide-water mixture as a special adaptation mechanism to the harsh Martian environment appears to be a plausible biochemical adaptation for Martian life to pursue given the environmental constraints (and would explain many of the Viking Life Detection observations). The hypothesis of life based on a H_2O-H_2O_2 internal solvent has significant implications for future missions, especially the Mars Phoenix Mission as it is scheduled to land in the northern latitude where living conditions for the hypothesized organisms should be close to ideal. Given the instrument suite on board of the Phoenix Mars Lander a bolder approach to include possible life detection measures should be considered.

References: [1] Cockell C.S. et al. (2005) *Astrobiology* 5, 127-140. [2] Diaz B. and Schulze-Makuch D. (2006) *Astrobiology* 6, 332-347. [3] Houtkooper J.M. and Schulze-Makuch D. (2006) Phys. Archives, http://arxiv.org/ftp/physics/papers/0610/0610093.pdf [4] Boynton W.V. et al. (2002) *Science* 297, 81-85. [5] Squires S.W. et al. (2004) *Science* 306, 1709-1714. [6] Baker V.R. et al. (1991) *Nature* 352, 589-594. [7] Dohm J.M. et al. (2001) *, J. Geophys. Res.106*, 32,943-32,958. [8] Fairén A.G. et al. (2003) *Icarus 165*, 53-67. [9] Schulze-Makuch D. et al. (2005) *J. Geophys. Res* 110, doi:10.1029/2005JE002430. [10] Schulze-Makuch D. And Houtkooper J.M. (2007) AAS Meeting, Seattle, WA, Pres. # 035.03. [11] Eschenbach D.A. et al. (1989) *J. Clin. Microbiol*. 27, 251-256. [12] Ryan C.S. and Kleinberg I. (1995) *Arch. Oral. Biol*. 40, 753-763. [13] Tanenbaum S.W. (1956) *Biochim. Biophys. Acta* 21, 335-342. [14] Sundaresan M. et al. (1995) *Science* 270, 296-299. [15] Rhee S.G. (2006) *Science* 312, 1882-1883. [16] MEPAg Special Regions-Science Analysis Group (2006) *Astrobiology* 6, 677-732. [17] Haberle R.M. et al. (2001) *J. Geophys. Res.*106, 23317-23326.