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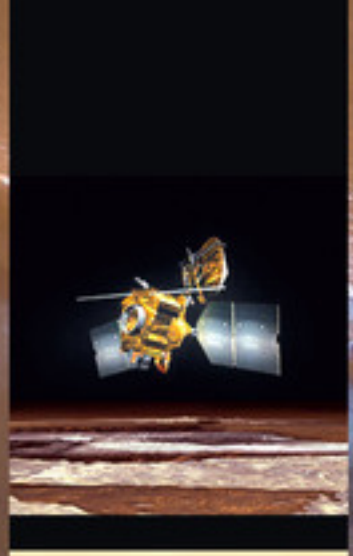

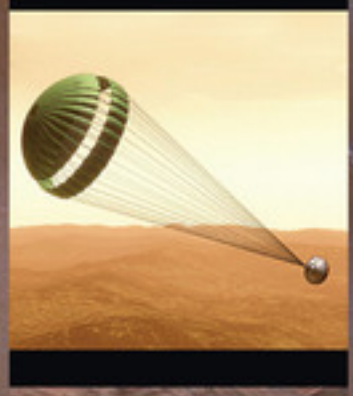

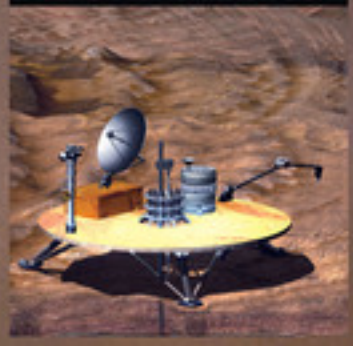

PREVENTING THE FORWARD CONTAMINATION OF MARS

PREVENTING THE FORWARD CONTAMINATION OF MARS

Committee on Preventing the Forward Contamination of Mars

Space Studies Board
Division on Engineering and Physical Sciences

NATIONAL RESEARCH COUNCIL
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Preface

Mars has been called "the most nearly similar to Earth of all the planets and one of the most likely repositories for extraterrestrial life among them."[1] Its proximity to Earth and its moderate climate make the planet more accessible for study than others in the solar system. The Viking lander missions in the 1970s explored two locations on Mars that suggested a dry, barren environment hostile to life.[2] However, recent spacecraft and robotic probes to Mars, including the Mars Global Surveyor, Mars Odyssey, the twin Mars Exploration Rovers Spirit and Opportunity, and the European Mars Express mission, have yielded a wealth of data that are significantly changing our understanding of the planet. Mars is now recognized as a heterogeneous planet of multiple environments, some of which might offer conditions suitable for extant or past life. In addition, studies of biology in extreme environments continue to expand the known range of environmental parameters compatible with life, and life-detection techniques have become ever more sensitive, enhancing the capabilities to find past or present life on the planet, should it exist. Indeed, the search for past and present life on Mars is the first of four nearly equal objectives in the Mars exploration strategy of the National Aeronautics and Space Administration (NASA).[3]

In light of these developments, the need to protect against contamination from Earth-borne organisms has become increasingly important. NASA thus requested that the National Research Council's (NRC's) Space Studies Board (SSB) examine existing planetary protection measures for Mars and recommend changes and further research to improve such measures.

Specifically, the Space Studies Board's Committee on Preventing the Forward Contamination of Mars accepted the following statement of task:

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[1]National Research Council, *Assessment of Mars Science and Mission Priorities*, National Academy Press, Washington, D.C., 2001, p. vii.

[2]National Research Council, *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan*, National Academy Press, Washington, D.C., 1978, pp. 3-13.

[3]"The overarching objectives for MEP [NASA's Mars Exploration Program] are: Life, Climate, Geology, and Preparation for Human Exploration. First among these objectives of nearly equal priority is Life." See MSPSG, *Mars Science Program Synthesis Group: Mars Exploration Strategy, 2009-2020,* D.J. McCleese, ed., JPL 400-1131, Jet Propulsion Laboratory, Pasadena, Calif., 2004, p. 4. In 2004, the Mars Exploration Program Analysis Group (MEPAG) endorsed the same four objectives but explicitly did not prioritize them. See MEPAG, *Scientific Goals, Objectives, Investigations, and Priorities: 2004*, unpublished document, available at <mepag.jpl.nasa.gov/reports/index.html>.

- Assess and recommend levels of cleanliness and bioload reduction required to prevent the forward contamination of Mars by future spacecraft missions (orbiters, atmospheric missions, landers, penetrators, and drills), given current understanding of the martian environment and of terrestrial microorganisms. The committee's recommendations should take into account the full spectrum of environments on, above, and under present-day Mars, and the various ways that spaceflight missions may access them, intentionally or inadvertently.
- Review methods used to achieve and measure the appropriate level of cleanliness and bioload reduction for Mars spacecraft and recommend protocol revisions and/or additions in light of recent advances in science and technology.
- Identify scientific investigations that should be accomplished to reduce the uncertainty in the above assessments.

The task specified that, to the maximum possible extent, the recommendations should be developed to be compatible with an implementation that would use the regulatory framework for planetary protection currently in use by NASA and the Committee on Space Research (COSPAR).

STUDY APPROACH AND PROCESS

The membership and qualifications of the Committee on Preventing the Forward Contamination of Mars are shown in Appendix A. The committee's work follows the NRC's previous advice to NASA on Mars planetary protection as provided in *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan* (NRC, 1978) and *Biological Contamination of Mars: Issues and Recommendations* (NRC, 1992); advice provided on the planetary protection of Europa in *Preventing the Forward Contamination of Europa* (NRC, 2000); advice provided in *Mars Sample Return: Issues and Recommendations* (NRC, 1997) on back contamination from samples collected on Mars and delivered to Earth; and advice in *Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies* (NRC, 1998) on samples returned from other solar system bodies. The recommendations relevant to the current study that were made in the 1992 and 2000 reports are summarized in Appendix B.

The committee explored a number of issues. It revisited arguments on the probability of contamination and the probability for the growth of Earth microorganisms on Mars as detailed in previous NRC reports, and it reevaluated that material in light of new knowledge. The committee took into account the question of liquid water on Mars; new knowledge about extremophilic microorganisms on Earth; new life-detection and bioburden-reduction techniques; the upcoming Mars Exploration Program; the potential for orbiter and lander crashes on Mars; the possible natural delivery of terrestrial microorganisms to Mars via meteorites launched from Earth; the implications for planetary protection of past spacecraft landings and crashes on Mars; and the COSPAR mission categories that are used to assign planetary protection requirements. It also discussed questions of the scope of planetary protection policy, including the protection of scientific investigations and the protection of the planet itself.

The committee held four meetings: a data-gathering meeting at the National Academies' Keck Center in Washington, D.C.; a mini-workshop at Diversa Corporation in San Diego, California; a writing meeting at the SETI Institute in Mountain View, California; and a subcommittee writing session at the National Academies' Beckman Center in Irvine, California. In addition, the committee held several teleconference calls to continue its deliberations and to discuss the draft report. In conducting its study, the committee considered input from several sources, including previous NRC reports as well as briefings and materials provided by NASA, the Jet Propulsion Laboratory, representatives from private industry, and the science and engineering community. In addition, the committee and meeting participants toured Diversa Corporation, a biotechnology company focused on cultivation-independent methods for recovery of and evolutionary studies on genes and biomolecules from the environment. One member of the committee visited a clean room for spacecraft assembly at the Jet Propulsion Laboratory to ascertain how planetary protection measures are implemented in practice, and two members visited associated research laboratories involved in advancing planetary protection techniques. Similarly, one committee member and staff visited the Lockheed Martin Astronautics Corporation to understand how the company has addressed

planetary protection for Mars spacecraft and the lessons, challenges, and issues involved in implementing planetary protection measures during spacecraft assembly.

ACKNOWLEDGMENTS

The committee acknowledges the many individuals who participated in and provided presentations at meetings: Peter Annan, Sensors and Software, Canada; Amy Baker, Technical Administrative Services; David Beaty, Jet Propulsion Laboratory (JPL); William Boynton, Lunar and Planetary Laboratory; Karen Buxbaum, JPL; Cathy Chang, Diversa Corporation; Benton Clark, Lockheed Martin Astronautics; James Garvin, NASA Headquarters; Bruce Jakosky, University of Colorado, Boulder; Robert Koukol, JPL; Brad Lobitz, San Jose State University Foundation; Gerald McDonnell, Steris Corporation; Brian Muirhead, JPL; Kenneth Nealson, University of Southern California; Laura Newlin, JPL; Roger Phillips, Washington University, St. Louis; John Rummel, NASA Headquarters; Andrew Spry, Open University, United Kingdom; Pericles Stabekis, The Windermere Group; Andrew Steele, Carnegie Institution; Kasthuri Venkateswaran, JPL; and Norman Wainwright, Marine Biological Laboratory.

Acknowledgment of Reviewers

This report has been reviewed by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC's) Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the authors and the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The contents of the review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their participation in the review of this report:

Michelle Alfa, University of Manitoba,
Philip R. Christensen, Arizona State University,
Edward F. DeLong, Massachusetts Institute of Technology,
Gerda Horneck, Institute of Aerospace Medicine, German Aerospace Center,
Bruce M. Jakosky, University of Colorado,
Jeffrey S. Kargel, U.S. Geological Survey,
Tullis Onstott, Princeton University,
David A. Stahl, University of Washington,
Peter Staudhammer, Alfred E. Mann Institute for Biomedical Engineering, and
James M. Tiedje, Michigan State University.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Mary Jane Osborn, University of Connecticut Health Center. Appointed by the National Research Council, she was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Contents

Executive Summary

The National Aeronautics and Space Administration's (NASA's) goals for space exploration over the coming decades place a strong priority on the search for life in the universe,[1] and the agency has set in place ambitious plans to investigate environments relevant to possible past or even present life on Mars. Over the next decade NASA plans to send spacecraft to search for evidence of habitats that may have supported extinct life or could support extant life on Mars; Europe will also send robotic explorers. These future missions, in addition to the ongoing suite,[2] will continue to deliver scientific data about the planet and reduce uncertainties about the prospects for past or present life on Mars. To ensure that scientific investigations to detect life will not be jeopardized, scientists have pressed, as early as the dawn of the space age, for measures to protect celestial bodies from contamination by Earth organisms that could hitchhike on a spacecraft, survive the trip, and grow and multiply on the target world.[3]

Preventing the forward contamination of Mars is the subject of this report, which addresses a body of policies, requirements, and techniques designed to protect Mars from Earth-originating organisms that could interfere with and compromise scientific investigations. The report does not assess forward contamination with respect to potential human missions to Mars, nor does it explore issues pertaining to samples collected on Mars and returned to Earth, so-called back contamination.[4] Those two dimensions of planetary protection, although extremely important, are beyond the scope of the charge to the Committee on Preventing the Forward Contamination of Mars. The recommendations made in this report do apply to one-way robotic missions that may serve as precursors to human missions to Mars. Included are recommendations regarding levels of cleanliness and biological burden on space-

[1]In its 2003 strategic plan, NASA cites as one of its goals "to explore the universe and search for life" (NASA, 2003). The Mars science community's Mars Exploration Program Analysis Group (MEPAG), in its 2004 report on scientific goals, objectives, investigations, and priorities for Mars exploration (MEPAG, 2004), and NASA's Mars Science Program Synthesis Group (MSPSG), in its published *Mars Exploration Strategy* (MSPSG, 2004), both identify the search for present and past life on Mars as one of four overarching goals of Mars exploration.

[2]NASA's current suite includes the Mars Exploration Rovers, Spirit and Opportunity, and the orbiters Mars Odyssey and Mars Global Surveyor; the European Space Agency's Mars Express is also in orbit.

[3]Letter from Joshua Lederberg, University of Wisconsin, to Detlev Bronk, President, National Academy of Sciences, December 24, 1957, with enclosed memorandum entitled "Lunar Biology?", National Academy of Sciences, Records Office, Washington, D.C.

[4]Back contamination, another aspect of planetary protection, involves the potential for contamination of Earth by any putative martian biota that might be returned to Earth on sample return missions.

craft destined for Mars, the methods employed to achieve those levels, and the scientific investigations needed to reduce uncertainty in preventing the forward contamination of Mars. In addition, this report urges dialogue at the earliest opportunity on broader questions about the role of planetary protection policies in safeguarding the planet Mars and an indigenous biosphere, should one exist.

In the United States, NASA has responsibility for implementing planetary protection policies that are developed in the international scientific community and, specifically, within the Committee on Space Research (COSPAR), a multidisciplinary committee of the International Council for Science (ICSU; formerly the International Council of Scientific Unions). COSPAR policies on planetary protection have evolved over time as scientists have acquired new information about Mars and other planets and about the potential for life to survive there. NASA has requested this National Research Council (NRC) study, and previous studies on the same topic from the NRC's Space Studies Board (SSB), to inform U.S. planetary protection practices; in turn, the NRC studies have provided input to the official COSPAR policies on planetary protection.

The committee evaluated current science about Mars, the ability of organisms to survive at the extremes of conditions on Earth, new technologies and techniques to detect life, methods to decontaminate and sterilize spacecraft, and the history and prior bases of planetary protection policy, as well as other relevant scientific, technical, and policy factors. It found that (1) many of the existing policies and practices for preventing the forward contamination of Mars are outdated in light of new scientific evidence about Mars and current research on the ability of microorganisms to survive in severe conditions on Earth; (2) a host of research and development efforts are needed to update planetary protection requirements so as to reduce the uncertainties in preventing the forward contamination of Mars; (3) updating planetary protection practices will require additional budgetary, management, and infrastructure support; and (4) updating planetary protection practices will require a roadmap, including a transition plan with interim requirements, and a schedule. In addition, the committee found that scientific data from ongoing Mars missions may point toward the possibility that Mars could have locales that would permit the growth of microbes brought from Earth, or that could even harbor extant life (although this remains unknown),[5] and that these intriguing scientific results raise potentially important questions about protecting the planet Mars itself, in addition to protecting the scientific investigations that might be performed there.

Taken together, the committee's recommendations constitute a roadmap for 21st-century planetary protection that emphasizes research and development; interim requirements; management and infrastructure for the transition to a new approach; and a systematic plan, process, and time line.

This executive summary presents a subset of the committee's recommendations. All of the committee's recommendations are included and discussed in Chapter 8.

RESEARCH AND DEVELOPMENT FOR 21st-CENTURY PLANETARY PROTECTION

For the most part, the bulk of NASA research and development on techniques to prevent the forward contamination of Mars was conducted during the Viking era, when the agency was preparing to send two landers to Mars that would include life-detection experiments.[6] Since the Viking program, continuing though comparatively little research has been done on planetary protection techniques, owing to the 20-year hiatus in Mars lander missions (Viking in 1976, Mars Pathfinder in 1996), the post-Viking perspective that Mars was a dry and barren place, and the expense and effort required to research, develop, and implement new requirements to prevent the forward contamination of Mars.[7]

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[5]See Chapters 4 and 5 and references therein.

[6]During the early 1970s, NASA undertook extensive research and development to better understand how to detect contamination on spacecraft and sterilize the spacecraft, and how methods used for those purposes would affect the spacecraft materials. The Viking mission was designed specifically with planetary protection in mind, which has not been the case for subsequent missions. See Bionetics Corporation (1990).

[7]See, for example, Dickinson et al. (2004a,b), Venkateswaren et al. (2001, 2003), Baker (2001), Baker and Rummel (2005), and Kminek and Rummel (2005).

The techniques currently available to detect contamination of spacecraft by microbes to some extent reflect the technologies that might be used to detect life on solar system bodies such as Mars. Life-detection techniques have advanced considerably, in part because of burgeoning biotechnology sciences and industries, allowing researchers the opportunity to employ molecular methods to identify the kinds and numbers of organisms that might be found in a spacecraft assembly area or on a spacecraft destined for Mars.

Knowledge about the diversity of organisms in clean rooms where spacecraft are assembled or on the spacecraft themselves has several important implications for planetary protection. At present, however, the standard assay method used for detecting microbes on spacecraft—a method that relies on detecting the presence of heat-resistant, spore-forming bacteria, which serve as a proxy for bioburden on the spacecraft—does not provide information about other organisms that might be present on spacecraft. Such organisms include the extremophiles—terrestrial organisms that survive and grow under severe conditions on Earth such as extremes of temperatures (hot and cold) and salinity, low availability of water, high levels of radiation, and other conditions previously considered hostile to life. Based on current understanding of Mars, it is thought that such organisms, especially the cold-loving ones (psychrophiles and psychrotrophs), are among those that might have the best chance of surviving and replicating in martian near-surface environments, as discussed in Chapter 5. Knowing specifically about the organisms present in assembly, test, and launch operations environments that might have the potential to survive a trip to, and possibly grow on, Mars would allow engineers to tailor methods to decontaminate a spacecraft and its instruments more effectively prior to launch than is now done. Other organisms with known intolerances for conditions much less severe than the harshness of interplanetary travel would be of less concern for preventing forward contamination, although efforts to clean[8] spacecraft would still be important for many missions.

A more tailored approach to bioburden reduction could also reduce the costs of implementing planetary protection as compared with the costs of existing approaches such as heat sterilization, which subjects a spacecraft, or specific parts of a spacecraft, to high temperatures over several hours in order to reduce the bioburden to the levels required by NASA for life-detection missions. Furthermore, heat sterilization, which was researched for and applied on the Viking mission in 1976, has not been tested for its effectiveness in eliminating extremophiles or other organisms now known to tolerate high heat. The committee therefore concluded that, ultimately, preventing the forward contamination of Mars requires an understanding of the kinds of organisms that could be present on spacecraft and sterilization or decontamination measures tailored to eliminate those organisms of concern.

To that end, the committee recommends a suite of research and development measures to enable updating of planetary protection practices to reflect the latest science and technology.

- **NASA should require the routine collection of phylogenetic data to a statistically appropriate level to ensure that the diversity of microbes in assembly, test, and launch operations (ATLO) environments, and in and on all NASA spacecraft to be sent to Mars, is reliably assessed. NASA should also require the systematic archiving of environmental samples taken from ATLO environments and from all spacecraft to be sent to Mars. (Recommendation 5, Chapter 8)**
- **NASA should sponsor research on those classes of microorganisms most likely to grow in potential martian environments. Given current knowledge of the Mars environment, it is most urgent to conduct research on psychrophiles and psychrotrophs, including their nutritional and growth characteristics, their susceptibility to freeze-thaw cycles, and their ability to replicate as a function of temperature, salt concentration, and other environmental factors relevant to potential spaceflight and to martian conditions. (Recommendation 6, Chapter 8)**
- **NASA should ensure that research is conducted and appropriate models developed to determine the embedded bioburden (the bioburden buried inside nonmetallic spacecraft material) in contemporary and future spacecraft materials. Requirements for assigned values of embedded bioburden should be updated as the results of such research become available. (Recommendation 7, Chapter 8)**

[8]"Cleaning" refers to reducing any nonliving contaminants of concern as well as living contaminants. Decontamination, bioburden reduction, and sterilization refer to standard methods that have proven to reduce the presence of bacterial spores to quantifiable levels. (See Chapter 2 for details.)

• NASA should sponsor studies of bioburden reduction techniques that are alternatives to dry-heat sterilization. These studies should assess the compatibility of these methods with modern spacecraft materials and the potential that such techniques could leave organic residue on the spacecraft. Studies of bioburden reduction methods should also use naturally occurring microorganisms associated with spacecraft and spacecraft assembly areas in tests of the methods. (Recommendation 8, Chapter 8)

• NASA should sponsor research on nonliving contaminants of spacecraft, including the possible role of propellants for future Mars missions (and the potential for contamination by propellant that could result from a spacecraft crash), and their potential to confound scientific investigations or the interpretation of scientific measurements, especially those that involve the search for life. These research efforts should also consider how propulsion systems for future missions could be designed to minimize such contamination. (Recommendation 9, Chapter 8)

• NASA should take the following steps to transition toward a new approach to assessing the bioburden on spacecraft:

—Transition from the use of spore counts to the use of molecular assay methods that provide rapid estimates of total bioburden (e.g., via limulus amebocyte lysate (LAL) analysis) and estimates of viable bioburden (e.g., via adenosine triphosphate (ATP) analysis). These determinations should be combined with the use of phylogenetic techniques to obtain estimates of the number of microbes present with physiologies that might permit them to grow in martian environments.

—Develop a standard certification process to transition the new bioassay and bioburden assessment and reduction techniques to standard methods.

—Complete the transition and fully employ molecular assay methods for missions to be launched in 2016 and beyond. (Recommendation 11, Chapter 8)

INTERIM REQUIREMENTS FOR USE UNTIL R&D EFFORTS ARE COMPLETE

Until the above-recommended R&D activities have been completed, the committee believes that the existing framework for planetary protection methods should be updated to reflect recent science regarding environments on Mars and knowledge about extremophiles. There is too much new information about the planet and new science about microorganisms not to update the existing framework of planetary protection requirements while research efforts are being conducted.

The most critical issue regarding Mars science and the potential forward contamination of Mars concerns so-called special regions. A "special region" is defined by COSPAR planetary protection policy as being "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms" (COSPAR, 2003, p. 71). Under existing COSPAR policy, missions to Mars are categorized as IVa (those without life-detection instruments), IVb (those with life-detection instruments),[9] or IVc (those going to special regions, regardless of instrumentation), and COSPAR policy sets levels of bioburden reduction differently for missions categorized as IVa, IVb, or IVc. Missions categorized as IVa are allowed higher levels of bioburden than missions that will carry life-detection instruments (IVb) or missions going to special regions (IVc).

The committee found, as discussed in Chapter 4, that there is at this time insufficient data to distinguish confidently between "special regions" and regions that are not special. Scientific results from the Mars Exploration Rovers and Orbiter missions have provided evidence for the existence of past water on Mars and suggest that it is substantially more likely that transient liquid water may exist near the surface at many locations on Mars. It is very difficult on the basis of current knowledge to declare with confidence that any particular regions are free of this possibility. Additional information is needed to identify the presence of liquid water, and collection of such data should continue to be a high priority.

[9]The previous NRC report on forward contamination, *Biological Contamination of Mars: Issues and Recommendations* (NRC, 1992), recommended the categories of Mars missions with life-detection instruments and those without life-detection instruments. A third category, special regions, was added to the COSPAR classification scheme in 2002.

• NASA's Mars Exploration Office should assign high priority to defining and obtaining measurements needed to distinguish among special and nonspecial regions on Mars. (Recommendation 10, Chapter 8)

The committee developed a new set of categorizations for Mars missions, IVs (missions to special regions) and IVn (missions not going to special regions). In the absence of sufficient data to distinguish IVs from IVn, the committee recommends that all landed missions to Mars be treated as IVs until additional data indicate or allow otherwise.

• For the interim period until updated planetary protection methods and techniques can be fully implemented,

—NASA should replace Categories IVa through IVc for Mars exploration with two categories, IVn and IVs. Category IVs applies to missions that are landing or crashing in, or traversing, excavating, or drilling into, special regions; Category IVn applies to all other Category IV missions.

—Each mission project should (in addition to meeting the requirements imposed by Categories IVn and IVs) ensure that its cleanliness with respect to bioburden and nonliving contaminants of concern is sufficient to avoid compromising its experiments, in consultation with NASA's planetary protection officer. (Recommendation 12, Chapter 8)

• Until measurements are made that permit distinguishing confidently between regions that are special on Mars and those that are not, NASA should treat all direct-contact missions (i.e., all Category IV missions) as Category IVs missions. (Recommendation 13, Chapter 8)

In addition to the issue of special regions, the committee analyzed several other issues pertinent to Category IV missions, including the kinetics of the growth of microorganisms that could potentially reproduce on Mars, the possibility of long-lived water, the probability of a mission crash, and the potential for radioisotope thermal generators (RTGs) to create liquid water. Based on this analysis, the committee devised five levels of bioburden reduction for application to Mars missions (see Table 8.1, Chapter 8).

• NASA should ensure that all category IVs missions to Mars satisfy at least level 2 bioburden reduction requirements.[10] For each Category IVs mission, NASA's planetary protection officer should appoint an independent, external committee with appropriate engineering, martian geological, and biological expertise to recommend to NASA's planetary protection officer whether a higher level of bioburden reduction is required. This analysis should be completed by the end of Phase A (performance of the concept study) for each mission. (Recommendation 14, Chapter 8)

[10]In Chapter 8, the committee defines level 2 as corresponding to the Viking-level pre-sterilization required for the bulk spacecraft plus Viking post-sterilization for all exposed surfaces; the latter is to be understood as an areal (surface density) measurement. Explicitly, Viking post-sterilization levels correspond to a reduction of 1×10^{-4} times the Viking pre-sterilization upper limit of 300 spores per square meter. Level 2 requirements (see Table 8.1) are not identical to those previously applied to Category IVs missions (Table 2.2), as is readily seen by comparing Tables 8.1 and 2.1. The committee also draws a distinction between mission categorization (based on mission destination) and bioburden reduction levels; e.g., Category IVs missions will typically be level 2 missions, but under some circumstances a decision could be made to require level 3 or higher for a particular Category IVs mission.

Note added in proof—The following text changes were approved and made after release of the prepublication copy of this report: (1) The phrase "in consultation with NASA's planetary protection officer" was added to Recommendation 12. (2) In Recommendation 14, the word "determine" was replaced by the phrase "recommend to NASA's planetary protection officer." (3) Two new sentences ("Level 2 requirements . . . for a particular Category IVs mission") were added to footnote 10.

MANAGING THE TRANSITION TO NEW PLANETARY PROTECTION POLICIES AND PRACTICES

Transitioning NASA's planetary protection practices to reflect current scientific understanding of Mars and advances in microbiology and to benefit from advanced technologies will require investments in a series of research and development efforts and assessments of new technologies that can be applied to the implementation of planetary protection policies. A successful transition will also depend on an infrastructure for managing these research efforts and on coordination with the engineering, spacecraft/instrument development, and science communities at NASA headquarters, NASA centers, industry, universities, research laboratories, and with the international community, especially COSPAR.

The committee recognizes that the research activities it recommends have cost implications. But it points out that the search for past and present life is cited as the second of NASA's 18 strategic objectives (NASA, 2005), and the attention to identifying potential habitats for life on Mars is reflected in the ambitious series of missions comprised by the Mars Exploration Program. Additional resources for updating planetary protection practices are critical for ensuring the integrity of these important scientific investigations. Such an investment could also introduce innovation into the planetary protection process, such as advanced technologies and methods that could potentially lead to faster and more effective practices for assessing and reducing the bioburden on Mars-bound spacecraft.

- **NASA should establish and budget adequately for, on an ongoing basis, a coordinated research initiative, management capability, and infrastructure to research, develop, and implement improved planetary protection procedures. The research initiative should include a training component to encourage the growth of national expertise relevant to planetary protection. (Recommendation 2, Chapter 8)**

In addition, recognizing the rapid advances in scientific understanding being gained from existing Mars missions and anticipated as a result of future missions, advances in life-detection technologies, and growth in research on and understanding of extremophiles, the committee concluded that NASA's planetary protection practices should be revisited on a 3-year basis to allow regular updates, as necessary.

- **NASA should establish an independent review panel that meets every 3 years to (1) consider the latest scientific information about Mars, as well as about Earth microorganisms, and recommend to NASA appropriate modifications to NASA's planetary protection implementation requirements as needed in light of new knowledge; and (2) identify and define the highest-priority measurements needed at Mars to inform future assessments and possible modifications of planetary protection requirements. (Recommendation 4, Chapter 8)**

The first meeting of the review panel should be held in 2008, and meetings should occur every 3 years thereafter, unless major changes in understanding of Mars or other factors related to planetary protection require meetings on an urgent basis.

RECONSIDERING PLANETARY PROTECTION: PROTECTING THE SCIENCE AND PROTECTING THE PLANET

Historically, planetary protection policy has addressed the concern that the forward contamination of planetary environments by terrestrial organisms could compromise current or subsequent spacecraft investigations sent to search for indigenous life. As a result, current practice imposes the strictest standards of cleanliness on those spacecraft that will conduct life-detection experiments, whereas spacecraft that will not search for life are required to meet less stringent standards. Nevertheless, current practice recognizes that missions intended to access "special regions" on Mars must comply with stricter standards, regardless of whether they carry life-detection instruments.

As discussed in Chapter 4, recent discoveries suggest that there may be numerous (and potentially difficult to detect) environments on Mars where the potential for terrestrial organisms to grow is substantially higher than

previously thought. For that reason, the committee recommends increased requirements for bioburden reduction until the results of new research and development make it possible to reduce the uncertainty in preventing the forward contamination of Mars. There remains the potential that lower standards of bioburden reduction permitted for spacecraft that do not include life-detection experiments may permit the introduction of terrestrial organisms into sensitive environments where they may reproduce over long time scales—posing a potential long-term threat to any indigenous biosphere that may exist.

Although ethical issues concerning the introduction of terrestrial organisms into sensitive extraterrestrial environments fall outside the mandate of the current committee, the committee believes that they should be given consideration at the earliest opportunity. The need for urgency in this deliberation is underscored by the current uncertainty regarding the extent and distribution of sensitive martian environments, the failure rate and cleanliness levels of past Mars landers, and the projected rapid pace of future spacecraft investigations. For these reasons, the committee recommends that NASA and its international partners address this issue as expeditiously as possible.

- **In light of new knowledge about Mars and the diversity and survivability of terrestrial microorganisms in extreme environments, NASA should work with COSPAR and other appropriate organizations to convene, at the earliest opportunity, an international workshop to consider whether planetary protection policies for Mars should be extended beyond protecting the science to include protecting the planet. This workshop should focus explicitly on (1) ethical implications and the responsibility to explore Mars in a manner that minimizes the harmful impacts of those activities on potential indigenous biospheres (whether suspected or known to be extant), (2) whether revisions to current planetary protection policies are necessary to address this concern, and (3) how to involve the public in such a dialogue about the ethical aspects of planetary protection. (Recommendation 1, Chapter 8)**

PLANETARY PROTECTION IN THE 21st CENTURY: A ROADMAP

The committee urges that its recommendations be considered as a roadmap; the recommendations build on each other to outline a modern planetary protection regime. (See Figure ES.1.) The committee also encourages NASA to implement these recommendations according to a transition plan and time line, as illustrated in Figure ES.2.

TRANSITION PLAN, PROCESS, AND TIME LINE

Given the rapid advancement in in situ science instrument capabilities and the possibility of contamination in a Mars environment potentially more water-rich than previously believed, it is important to review and adjust Mars forward contamination requirements and procedures as expeditiously as possible.[11] That said, the earliest chance to alter planetary protection procedures for Mars and begin to demonstrate, verify, and validate new methods from the ground up would likely be on the next new (not yet in development) flight project, i.e., the 2011 Mars Scout mission. The next program-directed mission, possibly a Mars Sample Return mission to be flown in 2013, will probably also begin its development at the same time as the 2011 Mars Scout mission, because it is expected to be a more complex mission and to require more development time before launch. Hence, there will be an opportunity during Fiscal Year 2008, when development of both the 2011 and the 2013 Mars missions is expected to start, to begin to test and demonstrate the effectiveness of new bioburden reduction requirements and procedures. Implementation of a new, completely validated planetary protection protocol that employs advanced bioassay and bioburden reduction methods would more realistically be accomplished on a mission developed for launch early in 2016. Such a transition would have to be initiated no later than the beginning of Fiscal Year 2012.

A set of four objectives for development of a new planetary protection plan and a schedule based on these

[11] A FY 2006 start date for the committee's recommended time line could depend on NASA's ability to access or reprogram resources to devote to research efforts.

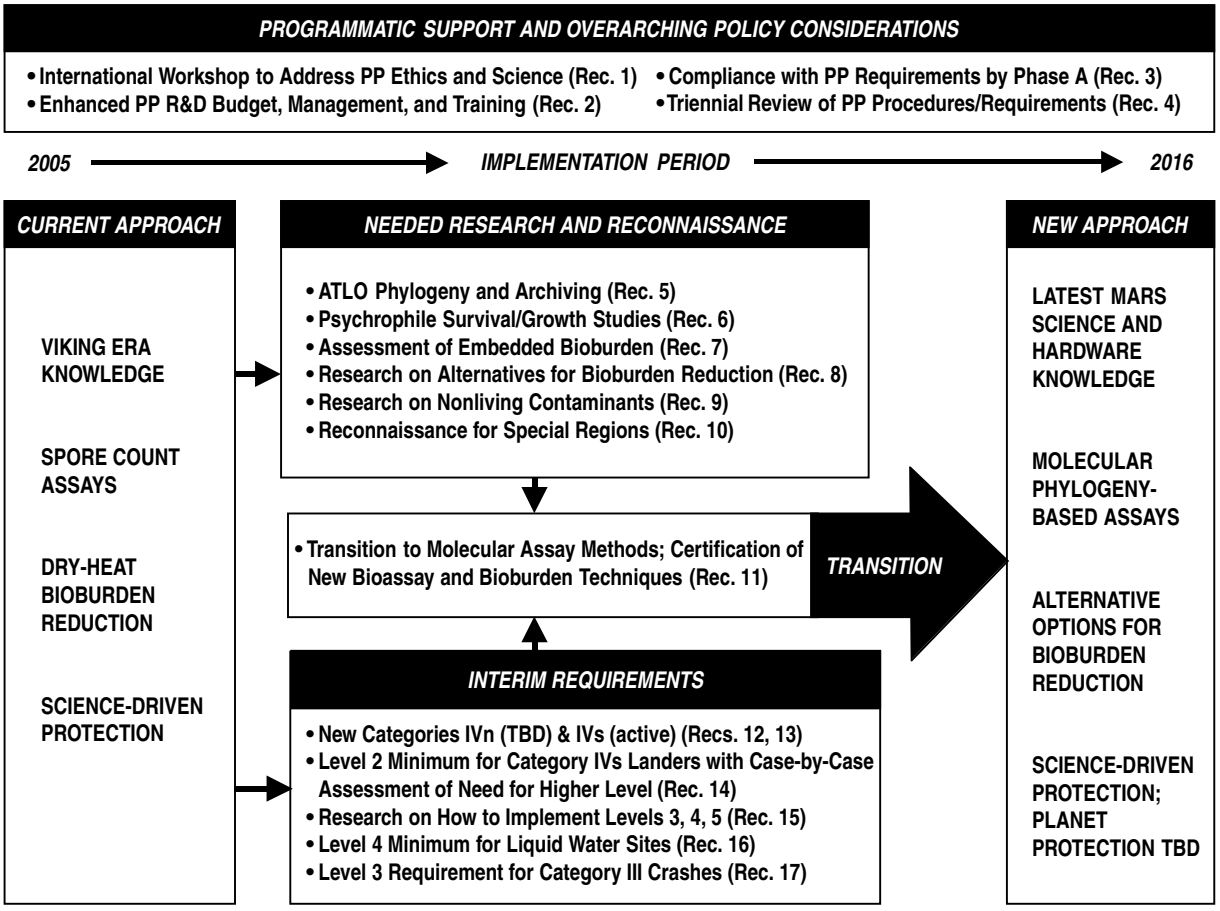
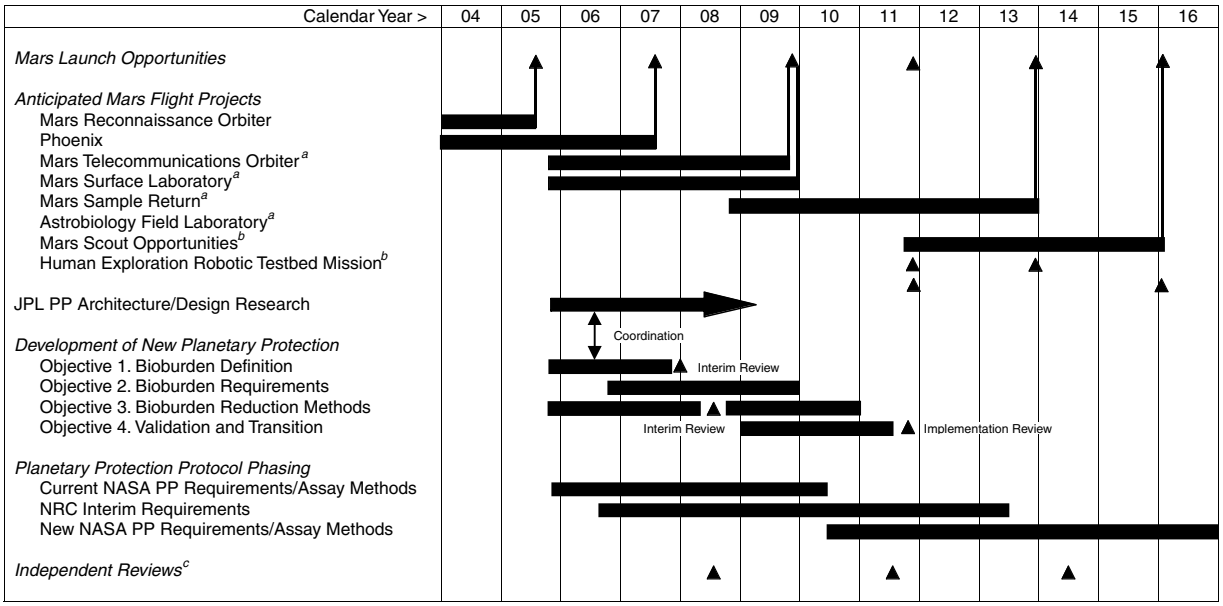


FIGURE ES.1 The proposed framework for moving from the current approach to the new approach to planetary protection (PP), along with the programmatic support and overarching policy considerations required to make the transition.

considerations, along with the development periods for all current and planned missions through the 2016 launch, is depicted in Figure ES.2. The four objectives are as follows:

- *Objective 1: Assessment of spacecraft contaminants.* The first step is to determine what microbes present in the construction, testing, and launch of Mars missions actually constitute potential threats either to Mars science or for contamination of the planet Mars itself. Assaying to know exactly what constitutes the bioburden within spacecraft development, assembly, and test facilities should be followed by determining what fraction of this bioburden could actually threaten to contaminate the Mars environment or confound planned life-detection measurements.
- *Objective 2: Definition and development of revised requirements for reduction of bioburden.* Review and revision of existing standards for reduction of bioburden (specifications), in terms of both parameters and limits, would follow from the ability to expressly target those microbial populations of greatest concern as potential contaminants (objective 1).
- *Objective 3: Improvement of bioburden reduction techniques.* Alternative bioburden reduction techniques could offer more effective and/or less stressful means of reducing or eliminating species-specific bioburdens. Knowing where and what bioburden must be reduced is necessary to determining when and how bioburden reduction can be accomplished and maintained throughout the mission development process.



[a]Flight projects in concept definition stage subject to changes in definition and launch date.
[b]Mars program elements with repetitive flight projects (opportunities shown are placeholders).
[c]Triennial reviews of Mars knowledge base, program plans, and planetary protection requirements (recommendation 4).

FIGURE ES.2 Proposed schedule for the revision of Mars planetary protection requirements.

- *Objective 4: Validation of and transition to new standards and techniques.* Changes in planetary protection practices enabled by meeting objectives 1 through 3 and proposed in response to the committee's recommendations must be validated. Hence new practices should be demonstrated and tested during a validation period in which existing bioburden reduction requirements continue to apply. Once validated and certified, new practices can then be applied with the confidence that they will provide the benefits expected, and old approaches can be phased out.

A complete transition to applying modern methods (without concurrent application of existing bioassay and bioburden reduction techniques) would most realistically be accomplished on a mission developed for launch early in 2016. Such a transition would have to be initiated no later than the beginning of Fiscal Year 2012. Assuming that a detailed research plan embracing the objectives outlined above is developed, reviewed, and funded within the next few years, NASA could accomplish the first three objectives outlined above within the next 6 years, as suggested in the proposed timeframe shown in Figure ES.2. Addressing the four objectives in the committee's recommended approach to updating planetary protection is an effort that clearly should be coordinated with a planned research effort at the Jet Propulsion Laboratory (JPL) (shown in Figure ES.2 as the JPL Planetary Protection Architecture/Design Research component) that shares several of the objectives of the approach outlined here. At NASA's discretion, this JPL work could even be integrated with the approach and schedule suggested here.

Because the results of each objective discussed above feed into and affect the subsequent objectives, periodic review of research progress by an independent panel is strongly recommended. As a separate matter, the committee recognizes that there would be an important interface to maintain with COSPAR to gain concurrence on a process that would clearly change how NASA complies with internationally acceptable planetary protection protocol.

The objectives for updating Mars planetary protection clearly illustrate that changing NASA's current approach to embrace advances in microbiology and growing understanding of Mars cannot be done quickly. Even an aggressive plan such as that outlined here will take the better part of a decade to complete and fully apply to the Mars Exploration Program. There is, therefore, every reason to begin the work at hand as quickly as possible.

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Introduction

In its 2003 strategic plan the National Aeronautics and Space Administration (NASA) cites as one of its goals "to explore the universe and search for life" (NASA, 2003). The Mars science community's Mars Exploration Program Analysis Group (MEPAG), in its 2004 report on scientific goals, objectives, investigations, and priorities for Mars exploration (MEPAG, 2004), and NASA's Mars Science Program Synthesis Group (MSPSG), in its published *Mars Exploration Strategy* (MSPSG, 2004), both identify the search for present and past life on Mars as one of four overarching goals of Mars exploration. The scientific community and NASA have thereby endorsed the investigation of the hypothesis that life may exist on Mars or may have existed previously, and they have made testing this hypothesis one of Mars exploration's primary goals.[1] As stated in NASA's Mars exploration strategy, "NASA is currently pursuing an aggressive, science-driven agenda of robotic exploration of Mars, with the aim of concluding the current decade of research with the first landed analytical laboratory on the martian surface since the Viking missions of the 1970s. This mobile science laboratory will propel Mars exploration into the next decade for which the search for evidence of biological activity is the ultimate goal" (MSPSG, 2004, p. 1).

This search necessarily brings with it the requirements of planetary protection. Planetary protection depends on a set of policies and practices designed to prevent the contamination of celestial bodies by terrestrial microorganisms that could hitchhike on a spacecraft, survive the trip, and grow and multiply on a planet, moon, asteroid, or comet—forward contamination—and to prevent the potential for any putative extraterrestrial biota that might be returned to Earth on sample return missions to contaminate Earth—back contamination. Preventing the forward contamination of Mars is the subject of this report.

The possibility of such planetary cross-contamination via spacecraft cannot be easily dismissed; experiments with bacterial spores on the European Retrievable Carrier (EURECA) and NASA's Long Duration Exposure Facility (LDEF) space missions (Horneck et al., 1994, 1995) have demonstrated that spores[2] of *Bacillus subtilis* survived 1 year in space at the 25 percent level and 6 years in space at the 1 percent level, respectively, provided

[1]Similarly, President George W. Bush's "New Vision for Space Exploration," announced January 14, 2004, states that the robotic exploration of Mars is to be conducted "to search for evidence of life, to understand the history of the solar system, and to prepare for future human exploration." See President's Commission (2004). See also NASA (2004).

[2]A spore is a tough, dormant form of certain bacterial cells that is especially resistant to desiccation, heat, and radiation. Spore-forming bacteria are common on Earth, but the vast majority of microorganisms are not spore formers.

they were shielded from solar ultraviolet light, as would be the case inside a spacecraft. Typical Earth-Mars spacecraft trajectories take less than 1 year.

Spacecraft assembled within highly controlled class-100,000 clean rooms have bacterial spore densities of ~10^3 spores per square meter on their surfaces (Barengoltz, 2004).[3] Thus, it is virtually certain that, in the absence of special measures, a large number of still-viable microbes will be present on interior spacecraft surfaces at the time a spacecraft reaches Mars from Earth. The focus then shifts to whether there are environments on Mars in which such organisms might survive and reproduce, whether these environments will be accessed by the spacecraft, and the likelihood and implications of varying answers to these questions. Planetary protection policy addresses these issues and the measures that should be taken in response.

POLICY BASIS FOR PLANETARY PROTECTION

The idea of planetary protection emerged with the genesis of the space program. Scientific leaders were the early proponents of planetary protection,[4] and in 1958 the U.S. National Academy of Sciences (NAS) passed a resolution stating, "The National Academy of Sciences of the United States of America urges that scientists plan lunar and planetary studies with great care and deep concern so that initial operations do not compromise and make impossible forever after critical scientific experiments."[5] The NAS resolution was brought to the International Council of Scientific Unions (ICSU, now known as the International Council for Science), which in 1958 created the ad hoc Committee on Contamination by Extraterrestrial Exploration (CETEX). CETEX met for about a year and provided the first guidance for planetary protection, including recommendations that interplanetary spacecraft be sterilized, and it further stated, "The need for sterilization is only temporary. Mars and possibly Venus need to remain uncontaminated only until study by manned ships becomes possible" (CETEX, 1959). CETEX also recommended that planetary protection be transferred to the newly formed multidisciplinary, international committee of the ICSU, the Committee on Space Research (COSPAR). COSPAR continues to serve as the international policy-making body on planetary protection, and it is a consultative body to the United Nations' Committee on the Peaceful Uses of Outer Space (Cypser, 1993).

Acting on the advice of its Consultative Group on Potentially Harmful Effects of Space Experiments, COSPAR in 1964 issued Resolution 26 (COSPAR, 1964, p. 26), which

> affirms that the search for extraterrestrial life is an important objective of space research, that the planet of Mars may offer the only feasible opportunity to conduct this search during the foreseeable future, that contamination of this planet would make such a search far more difficult and possibly even prevent for all time an unequivocal result, that all practical steps should be taken to ensure that Mars be not biologically contaminated until such time as this search can have been satisfactorily carried out, and that cooperation in proper scheduling of experiments and use of adequate spacecraft sterilization techniques is required on the part of all deep space probe launching authorities to avoid such contamination.

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[3]A class-100,000 clean room is defined as a clean room with 100,000 0.5-micron-diameter particles per cubic foot of atmosphere. Class-100,000 clean rooms typically require restricted access, positive pressurization, and perhaps other measures. Clean rooms with fewer atmospheric particles have more stringent requirements. Class-10,000 clean rooms are typical of hospital operating rooms; class-1,000 facilities are typical for making computer disk drives, and class-100 are typical for semiconductor and pharmaceutical manufacture. Class-10 and class-1 rooms also exist. Definitions of clean rooms are given in "Federal Standard 209E: Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones," available at <www.zenobi.ethz.ch/Analytik5/USstandard.pdf>. Federal Standard 209E has been formally superseded by International Organization for Standardization (ISO) Standards in Metric Units; see "Cancellation of Fed-STD-209E," available at <www.iest.org/publctns/fedstd209.htm>. However, U.S. usage still often refers to the imperial unit definitions. For a discussion of clean-room levels and requirements, see, for example, <www.dataclean.com>.

[4]Letter from Joshua Lederberg, University of Wisconsin, to Detlev Bronk, President, National Academy of Sciences, December 24, 1957, with enclosed memorandum entitled "Lunar Biology?", National Academy of Sciences, Records Office, Washington, D.C.

[5]National Academy of Sciences, resolution adopted by the Council of the NAS, February 8, 1958. Addendum to Minutes of the Meeting of the Council of the National Academy of Sciences, February 8, 1958.

THE OUTER SPACE TREATY

Language related to planetary protection was incorporated into Article IX of the Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, including the Moon and Other Celestial Bodies (known as the Outer Space Treaty), which entered into force in 1967:

> States Parties to the Treaty shall pursue studies of outer space, including the moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose.[6]

The United States signed and ratified the Outer Space Treaty in 1967, and so is legally bound by the treaty's requirement to avoid harmful contamination of the Moon and other celestial bodies. The treaty was the second so-called non-armament treaty of the Cold War and was in some respects modeled on its predecessor, the Antarctic Treaty, which entered into force in 1961 (U.S. Department of State, 2004). Article IX of the Antarctic Treaty called for states that are parties to the treaty to recommend measures to further "the preservation and conservation of living resources in Antarctica." Article IX of the Outer Space Treaty, however, is ambiguous with respect to whether its focus is on protecting celestial bodies themselves or the scientific interests of those countries exploring them.

A policy review of the Outer Space Treaty concluded that, while Article IX "imposed international obligations on all state parties to protect and preserve the environmental integrity of outer space and celestial bodies such as Mars," there is no definition as to what constitutes harmful contamination, nor does the treaty specify under what circumstances it would be necessary to "adopt appropriate measures" or which measures would in fact be "appropriate" (Goh and Kazeminejad, 2004, p. 219). An earlier legal review, however, argued that "if the assumption is made that the parties to the treaty were not merely being verbose" and "harmful contamination" is not simply redundant, "harmful" should be interpreted as "harmful to the interests of other states," and since "states have an interest in protecting their ongoing space programs," Article IX must mean that "any contamination which would result in harm to a state's experiments or programs is to be avoided" (Cypser, 1993, pp. 324-325). Both reviews, and their interpretations, are unofficial.

Current NASA policy states that the goal of NASA's forward contamination planetary protection policy is the protection of scientific investigations (Rummel and Billings, 2004), declaring explicitly that "the conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized" (NASA, 1999). This has been the approach taken by COSPAR for the past four decades, most obviously with respect to its idea of a finite "period of biological exploration" beyond which planetary protection measures need not extend.[7] Consistent with this approach, the protection of the ability to perform scientific measurements without confounding them with false positives has been the focus of past National Research Council (NRC) examinations of forward contamination planetary protection policy for Mars. In its 1992 report *Biological Contamination of Mars: Issues and Recommendations*, the Space Studies Board (SSB) Task Group on Planetary Protection emphasized that "the philosophical intent of the 1978 committee [the SSB committee that had previously addressed the topic] to protect Mars from terrestrial contamination so as not to jeopardize future life-detection experiments on Mars is still profoundly important" (NRC, 1992, p. 57).

The present committee's statement of task reads in part, "To the maximum possible extent, the [committee's] recommendations should be developed to be compatible with an implementation that will use the regulatory framework for planetary protection currently in use by NASA and COSPAR,"[8] which the committee understood as

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[6]The full text of the treaty, its membership, and related documents such as the Antarctic Treaty appear in Rauf et al. (2000), pp. 138-142 (treaty text) and pp. 241-247 (membership).

[7]The period of biological exploration is referred to as either a defined number of years or the time to completion of a series of robotic missions to, or experiments on, Mars, during which strict planetary protection practices must be followed to protect the planet for the conduct of scientific investigations, including the search for life.

[8]See the committee's statement of task, reproduced in the Preface to this report.

a call for it to restrict its formal recommendations, to the maximum extent possible and consistent with the mandate of previous SSB committees, to the goal of protecting current and future scientific investigations.

Scientific investigations could in principle be jeopardized either by biological contaminants carried on a spacecraft intended to perform certain life-detection or life-related experiments itself, or by the establishment and growth of terrestrial organisms on Mars that could then interfere with scientific investigations of subsequent missions to the planet. Certain life-detection techniques could also be jeopardized by the delivery to Mars of nonliving material, either remnants of organisms or, possibly, chemicals that were not biological in origin.

Because Mars and other celestial bodies are "not subject to national appropriation by claim of sovereignty, by means of use or occupation, or by any other means" (Article II of the Outer Space Treaty), planetary protection is inherently international and a matter of concern for all existing and future spacefaring nations.

PROTECTING SCIENCE AND PROTECTING MARS

The committee is aware that some in the scientific community have increasingly voiced concerns about ensuring the environmental integrity of other celestial bodies, aside from protecting scientific investigations for their own sake. In particular, the SSB Task Group on the Forward Contamination of Europa noted in its report *Preventing the Forward Contamination of Europa* that "future spacecraft missions to Europa must be subject to procedures designed to prevent its contamination by terrestrial organisms. This is necessary to safeguard the scientific integrity of future studies of Europa's biological potential and to protect against potential harm to europan organisms, if they exist, and is mandated by obligations under the [Outer Space Treaty]" (NRC, 2000, p. 13). That is, the Europa task group declared that in europan planetary protection policy, protection of europan organisms was as important as protection of scientific studies. Virtually identical concerns about protecting a possible europan biosphere had been articulated by the SSB Committee on Planetary and Lunar Exploration (NRC, 1999, p. 72) in its report *A Science Strategy for the Exploration of Europa*.

The present committee considered at length the importance of protecting scientific research at Mars for the period of biological exploration versus protecting the planet Mars, potentially in perpetuity. Although an assessment of the protection of planet Mars goes beyond the statement of task for this study, the committee was conscious of the fact that recent Mars exploration has revealed a planet that now appears more potentially hospitable to terrestrial microbes than was envisioned by the 1978 or 1992 NRC studies. Whether the predominant rationale for planetary protection is to protect the science or to protect the planet involves differing viewpoints and significant uncertainties that are likely to continue for many years. The committee considered both aspects of planetary protection, although the committee's statement of task, and thus its data gathering, deliberations, and conclusions, centered on the current COSPAR policy of protecting the planet for science.

Human Missions to Mars

In January 2004, President George W. Bush announced "A Renewed Spirit of Discovery: The President's Vision for U.S. Exploration," which set the goal of human exploration of the Moon, Mars, and the solar system.[9] That vision underscored the importance of the Mars robotic program as a testbed for demonstrating technological capabilities that are "key to enabling future human Mars missions." Although the committee's charge focuses on preventing forward contamination of Mars by varied future spacecraft missions and activities, "including orbiters, atmospheric missions, landers, penetrators, and drills,"[10] upcoming exploration by human missions seems likely in light of the national vision for space exploration put forth by President Bush and the subsequent Moon-Mars initiative (President's Commission, 2004; NASA, 2004). Future human or robotic-aided human missions are likely to present significant forward contamination challenges in both planning and implementation.

[9]See President's Commission on Implementation of the United States Space Exploration Policy (2004).
[10]See the charge to the committee in the Preface to this report.

Human missions will inevitably introduce considerations that go beyond those covered by the forward contamination controls and policies discussed in this report. Furthermore, they are likely to involve examination of COSPAR policies and questions about minimizing potential contamination that could be introduced through human operations, exploration, construction, sampling, and sequencing of activities. Today, there are no official COSPAR or NASA policies encompassing forward contamination of solar system bodies during human missions. Although significant study will be necessary before planning and implementing contamination controls for human missions, the committee recognizes that planetary protection considerations will be important in all phases of future missions, whether robotic or human. The committee notes that previous NRC reports—*Biological Contamination of Mars: Issues and Recommendations* (NRC, 1992) and *Safe on Mars: Precursor Measurements Necessary to Support Human Operations on the Martian Surface* (NRC, 2002)—have addressed human missions to Mars and have concluded that information from precursor robotic missions is critical for planning safe, productive human missions that will have a minimal impact on Mars.

In anticipating the long-term potential for expansion of human activities on Mars, it may be prudent to consider forward contamination policies in the context of analogous policies for sensitive environments on Earth, such as the international treaty governing Antarctica.[11] Like the Outer Space Treaty, the Antarctic Treaty calls for peaceful use for humanity, freedom of scientific investigations, and international cooperation. The Antarctic Treaty also specifically calls for the preservation and conservation of living resources in Antarctica. Examination of the administrative oversight and controls imposed on research and activities in polar areas, such as the designation of special regions, requirements for waste disposal and cleanup, and reversibility of human actions, may be useful in developing a framework for addressing concerns related to forward contamination by human missions.

The committee does not, however, take a position on whether human missions to Mars will or will not necessarily broadly contaminate the martian surface with terrestrial microorganisms—a topic that will require extensive study and possibly research and development (R&D).

Implementation of Planetary Protection

Translating planetary protection into actual practice involves a complex mix of intertwined policies, elements of science and engineering, uncertainties, and implementation protocols. For past robotic missions to Mars, forward contamination controls have included the requirement to reduce the biological contamination of the spacecraft, constraints on spacecraft operating procedures, and inventories of organic constituents of the spacecraft and organic samples, along with documentation of spacecraft operations, impact potential, and the location of landing or impact points on the planetary surface.

Upon request, the SSB has provided advice to NASA about planetary protection (see Appendix B). In 1978, the SSB produced *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan,* which considered planetary protection for several bodies (NRC, 1978). In 1992, the SSB issued *Biological Contamination of Mars: Issues and Recommendations* (NRC, 1992), while other reports have considered sample return and back contamination. SSB reports have provided scientific input that has been used to update COSPAR policies as well as practices that must be implemented to meet those policies.

COSPAR maintains and issues policy guidance on planetary protection to the international space science community. NASA and other national space agencies adhere to COSPAR planetary protection policy to avoid the contamination of extraterrestrial bodies. NASA's Planetary Protection Office provides the implementation requirements for planetary protection for NASA's planetary exploration program, including the Mars Exploration Program. The methods and practices of planetary protection developed during the 1970s for the Viking mission set the standard operating procedures that are still in use today. Current planetary protection practices and their historical development are discussed in detail in Chapter 2. Important terminology in the planetary protection lexicon is defined in Box 1.1.

[11]The Antarctic Treaty, December 1, 1959. For text, see Rauf et al. (2000), pp. 132-135. For a historical account of the treaty, see NRC (1993).

BOX 1.1
Planetary Protection Terminology

The practices of detecting, cleaning, and reducing the bioburden presented by microorganisms on spacecraft are described with a particular set of terms that is used in discussing planetary protection. Some of these practices and terms stem from the Viking Lander program, during which researchers conducted extensive studies on spacecraft cleaning methods and spacecraft sterilization that have continued to serve as the basis for planetary protection requirements (see Chapter 2). Other terms refer to probabilistic approaches that have been used in the past to establish requirements for the cleanliness of spacecraft.

Assay: an experimental analysis, usually involving sampling techniques, used to derive data on which to base an estimate of the number or kind of microorganisms associated with an item of interest.

Bioburden: level of microbial contamination (total number of microbes or microbial density) in or on an item of interest.

Bioburden reduction (also known as microbial reduction): reduction by any qualified process (temperature, chemical, radiative, or combinations thereof) of the number of organisms on spacecraft or components to a specified level.

Committee on Space Research (COSPAR): the international body responsible for formulating policies in accordance with the Outer Space Treaty; it is a committee of the International Council of Scientific Unions (ICSU; now called the International Council for Science).

Dry-heat cycle (also known as baking): the only NASA-certified method for reduction on an entire spacecraft and the preferred method for bringing spacecraft to sterile or near-sterile conditions; involves a heat cycle using prescribed temperature, pressure, gas, and humidity conditions for a specified length of time.

Encapsulated (embedded) bioburden: bioburden buried inside nonmetallic spacecraft material.

Forward contamination: contamination by biological or other organic material carried on outbound spacecraft to celestial bodies that may jeopardize the conduct of scientific investigations of possible extraterrestrial life forms, both extinct and extant.

PAST DELIVERY OF MICROORGANISMS TO MARS

The committee considered the implications for planetary protection requirements of past natural and mission-associated delivery of Earth microorganisms to Mars. An extreme viewpoint would be that because some past missions have already likely delivered significant quantities of microorganisms to Mars, and because Mars experiences substantial windblown transport of dust, there is no longer any point in continuing planetary protection practices.

All past missions that have landed or crashed on Mars (even the rigorously heat-sterilized Viking missions) have virtually certainly delivered some viable microorganisms to the martian surface. Table 1.1 displays the outcome of all missions of all nationalities sent from Earth to Mars; it also notes which of these missions failed and which crashed onto the martian surface. Soviet planetary protection measures were judged by the U.S. planetary protection officer in 1972 to "approximate compliance with COSPAR constraints," assuming that the Soviet space

Mated bioburden: microbial burden associated with spacecraft surfaces that have been joined with fasteners rather than adhesives (which embed bioburden when surfaces are joined) during the spacecraft assembly process.

Period of biological exploration: a period referred to as either a defined number of years or the time to completion of a series of robotic missions to, or experiments on, Mars, during which strict planetary protection practices must be followed to protect the planet for the conduct of scientific investigations, including the search for life.

Probability of contamination (P_c): the probability that a mission will contaminate a planet, calculated according to a formulaic approach based on measurements of bioburden at launch, combined with microorganisms' likely survival in space, release onto the planet, and growth in the new environment.

Probability of growth (P_g): the probability that a terrestrial microbe on a spacecraft delivered to an extraterrestrial body will grow and reproduce in that environment.

Special region: a specially designated region on Mars—currently defined by COSPAR as "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms." Currently applied to regions where liquid water is present or may occur.

Viking pre-sterilization: treatment to a level of cleanliness based on cleaning or sterilizing, or both, the spacecraft and its component parts in a manner such that the density of culturable microbial spores is less than $300/m^2$ on the spacecraft surface and the total number on the launched spacecraft is not greater than 3×10^5.

Viking post-sterilization: treatment to a level of cleanliness of an assembled spacecraft accomplished by using a final sterilization process (e.g., dry heat) to reduce the Viking pre-sterilization levels of microbes by 4 orders of magnitude, resulting in a total of no more than 30 culturable microbial spores on the surface of the launched spacecraft.

program "did, or will, carry out the measures described."[12] Neither the U.S. Pathfinder or Mars Polar Lander, nor the Mars Exploration Rover (MER) missions were subject to the dry-heat sterilization of the Viking missions. Missions that have crashed on the martian surface, such as the Mars Polar Lander mission launched in 1999, are likely not only to have exposed the martian environment to some interior surfaces, but also to have released some of their embedded bioburden, due to ruptures in spacecraft materials.

The likelihood of past delivery of spacecraft microbial material to Mars does not vitiate ongoing planetary protection measures. The prospects for the forward contamination of Mars (which requires microorganism survival

[12]Lawrence B. Hall, "Analysis of the Planetary Quarantine Effort in the U.S.S.R.," memorandum to Associate Administrator for Space Science [date obscured; believed to be January 1972, the date of an associated memorandum] and enclosures.

TABLE 1.1 History of Successes and Failures of Mars Missions

Mission	Type	Country	Launch Date	Mission Outcome		Comments
				Success	Failure	
Unnamed Mars Mission	Flyby	USSR	10-24-62		X	Interplanetary stage failure; main engine turbopump exploded
Mars 1	Flyby	USSR	11-01-62		X	Lost in space due to antenna pointing problem
Sputnik 24	Flyby	USSR	11-04-62		X	Launch sequence failure
Mariner 3	Flyby	USA	11-05-64		X	Launch sequence failure
Mariner 4	Flyby	USA	11-28-64	X		
Zond 2	Flyby	USSR	11-30-64		X	Flew by Mars without returning any data
Mariner 6	Flyby	USA	02-24-69	X		
Mariner 7	Flyby	USA	03-27-69	X		
Unnamed Mars Mission	Orbiter	USSR	03-27-69		X	Proton third-stage failure
Unnamed Mars Mission	Orbiter	USSR	04-02-69		X	Proton first-stage failure
Mariner 8	Orbiter	USA	05-08-71		X	Launch sequence failure
Kosmos 419	Orbiter	USSR	05-10-71		X	Upper stage failure
Mars 2	Orbiter/Lander	USSR	05-19-71		X	Successful entry, descent, landing (EDL), but crash landed without returning any data
Mars 3	Orbiter/Lander	USSR	05-28-71	X		Descent module instruments transmitted for 20 seconds after landing; then ceased transmitting
Mariner 9	Orbiter	USA	05-30-71	X		
Mars 4	Orbiter/Lander	USSR	07-21-73		X	Flew past Mars due to orbit rocket failure
Mars 5	Orbiter/Lander	USSR	07-25-73		X	Entered orbit but failed several days later
Mars 6	Orbiter/Lander	USSR	08-05-73		X	Successful EDL but failed with terminal rocket ignition
Mars 7	Orbiter/Lander	USSR	08-09-73		X	Failed due to pre-Mars separation of orbiter and lander
Viking Orbiter 1	Orbiter	USA	08-20-75	X		
Viking Lander 1	Lander	USA	08-20-75	X		
Viking Orbiter 2	Orbiter	USA	09-09-75	X		
Viking Lander 2	Lander	USA	09-09-75	X		
Mars Observer	Orbiter	USA	09-25-92		X	Probable failure of propellant line due to hypergolic propellant contamination
Mars Global Surveyor	Orbiter	USA	11-07-96	X		
Mars '96	Orbiter/Landers/ Penetrators	USSR	11-17-96		X	Launch sequence failure
Mars Pathfinder	Lander/Rover	USA	12-04-96	X		
Mars Climate Orbiter	Orbiter	USA	12-11-98		X	Disintegrated in the atmosphere due to navigation error
Mars Polar Lander	Lander	USA	01-03-99		X	Crashed due to premature shutdown of retrorocket engines
Mars DS-2 (renamed Amundsen)	Penetrator	USA	01-03-99		X	Unknown EDL failures; surface communication system malfunction suspected
Mars DS-2 (renamed Scott)	Penetrator	USA	01-03-99		X	Unknown EDL failures; surface communication system malfunction suspected

TABLE 1.1 Continued

Mission	Type	Country	Launch Date	Mission Outcome: Success	Mission Outcome: Failure	Comments
Mars Odyssey	Orbiter	USA	04-07-01	X		
Mars Express	Orbiter	ESA	06-02-03	X		
Beagle 2	Lander	UK	06-02-03		X	Unknown EDL failure; excessive impact velocity suspected
MER (Spirit)	Lander/Rover	USA	06-10-03	X		
MER (Opportunity)	Lander/Rover	USA	07-07-03	X		
	Total Successes/Failures			15	21	
	Overall Success Rate			42%		
	NASA Successes/Failures			13	7	
	NASA Success Rate			65%		
	NASA Lander Successes/Failures			5	3	
	NASA Lander Success Rate			63%		

SOURCES: Data for missions launched before 1992 were taken from NASA (1991), Siddiqi (2002), and <http://nssdc.gsfc.nasa.gov/database/MasterCatalog?sc=1971-049A>, accessed on November 15, 2005. Data for all subsequent missions were obtained from NASA Web sites.

and growth) are inherently probabilistic. Even if each previous mission did have some probability of having contaminated Mars, those probabilities were likely small (see Chapters 4 and 5), so that care with subsequent missions is still important for keeping at a low level the probability of contaminating Mars summed over all missions. The committee illustrates this concept with an analogy: even if the campfires of a dozen campers have previously posed the risk of a forest fire, it is still important that subsequent campers extinguish their campfires properly.[13]

Unless a previous mission has delivered microbes to an environment in which they can reproduce and geographically expand via the martian subsurface, existing experimental evidence for the survival of microorganisms at the surface of Mars suggests that the contamination resulting from these missions is likely to be at most local. More than 30 research papers have been published reporting experimental results for microbial survival under simulated martian conditions, with inconsistent results. Only recently have such experiments been conducted in a Mars simulation chamber that permitted good simulation of the pressure, temperature, atmospheric composition, and ultraviolet (UV)-visible-infrared light environment at Mars (Schuerger et al., 2003). These experiments showed that *B. subtilis* spores were rapidly (timescales of hours at most) killed even when partly shielded against UV light by being covered with simulated martian dust particles up to 50 microns in diameter. Viable spores were significantly reduced after an 8-h period even when covered by a 0.5-mm contiguous dust layer. Experiments with the dessication-tolerant, endolithic cyanobacterium *Chroococcidiopsis sp.* 029 (Cockell et al., 2005) showed survival for this organism, when exposed to martian-simulated UV, about 10 times higher than that previously reported for *B. subtilis*, but there was still a 99 percent loss of cell viability after 5 minutes. However, if protected by 1 mm of rock, *Chroococcidiopsis sp.* could survive and potentially grow, if water and nutrient requirements for growth were met.

It appears likely that most microorganisms exposed to the martian UV environment and unable to gain access to the martian subsurface will rapidly die. Moreover, because windblown dust particles on Mars have diameters in the range of 1 to 2 microns, transport via dust particles is also likely to lead to rapid death, and so windblown transport of microorganisms on Mars seems unlikely to contaminate distant parts of Mars.

[13]The committee called this the "Smokey the Bear" argument for ongoing planetary protection.

In addition, the committee considered that Mars and Earth likely exchange meteorites in a size range sufficiently large to protect microorganisms against exposure to solar UV and some cosmic rays during travel in interplanetary space, but small enough to allow soft landings after deceleration in the atmosphere.[14] A careful though necessarily speculative treatment of this problem suggests that ~10^{11} to 10^{12} viable bacteria may have been delivered to Mars in such Earth-originating meteorites (Mileikowsky et al., 2000). Some extremely small fraction of such meteorites is expected to complete the Earth-Mars trajectory within years, but most will take ~10^5 to 10^6 years for the journey. While the estimates of Mileikowsky et al. (2000) for total viable bacteria delivered to Mars endeavor to account for mortality over these long timescales, the estimates necessarily represent extrapolations of 4 to 5 orders of magnitude beyond actual data (obtained from the Long Duration Exposure Facility or European Retrievable Carrier) for survival of microorganisms in the space environment. Moreover, delivery by meteorite to one or another spot on the martian surface is not the same as delivery as a result of a spacecraft landing intended to access martian regions where liquid water is especially likely to exist. The search for liquid water is in fact a high priority in current Mars exploration (see Chapter 3).

Despite past mission-associated and natural delivery of microorganisms to Mars, the committee concluded that the challenge of planetary protection cannot be put aside in upcoming Mars exploration. The remainder of this report first gauges the magnitude of that challenge and then recommends how NASA should address it.

ISSUES IN AND ORGANIZATION OF THIS REPORT

Chapter 2 presents a detailed account of current planetary protection policies and policy implementation, establishing a baseline of current practices against which the requirements of future exploration can be measured. There are four broad reasons that current policies should be reconsidered and updated: (1) an extensive planned series of missions to Mars, (2) new information about the surface of Mars relative to life, (3) new findings regarding microorganisms on Earth, and (4) advances in technologies relevant to life detection and bioburden reduction that make improved approaches possible.

Chapter 3 describes the planned Mars exploration strategy now envisioned for the coming decades. These missions will target regions (including subsurface regions) of Mars that are especially likely to harbor liquid water, an approach that emphasizes the growing challenges that the "rolling wave" of upcoming missions could pose for planetary protection.

Based largely on Mars exploration missions to date, scientific understanding of Mars and the prospects for liquid water and potentially habitable environments there is evolving rapidly. Questions regarding the environments and conditions in which liquid water may be present on Mars, which are discussed in Chapter 4, are central to the prospects for and potential habitats of life on Mars, as well as the prospects for forward contamination.

Prospects for the forward contamination of Mars depend both on the nature of the Mars environment and on the conditions in which Earth microorganisms can survive and grow. Chapter 5 describes the rapid growth in knowledge of the limits of life on Earth, with emphasis on the state of scientific research on microbial survival in Earth environments that in some ways approximate those that may exist on Mars.

Chapter 6 describes the new molecular technologies that now permit a far greater understanding of the numbers and nature of microorganisms present on spacecraft bound for Mars. Many of these technologies have been proposed recently for use in detecting life in Antarctica, one proposed Earth analog to Mars (NRC, 2003). The application of modern techniques to assaying spacecraft bioburden would permit planetary protection measures to focus on the small number of microorganisms of greatest concern. New methods can also be applied to limit the viable bioburden on spacecraft before launch. These methods are crucial for evaluating the prospects for the future

[14]The committee notes, however, that the "contamination" of concern in planetary protection (see Chapter 1) refers strictly to contaminants carried on spacecraft. The natural exchange between Earth and Mars of microorganisms in meteorites, whatever its magnitude, does not constitute "contamination" from the point of view of planetary protection. [*Editor's note*—This footnote was approved and added after release of the prepublication copy of this report.]

application of either more rigorous or more selective bioburden reduction techniques. Such potential new methodologies for bioburden reduction are also discussed in Chapter 6.

Many of the same molecular detection technologies that will permit development of a detailed understanding of spacecraft bioburden can also be used to conduct extremely sensitive searches for life on Mars. Some of these searches could be confounded by nonliving terrestrial contaminants, such as the remains of dead microorganisms that were carried on spacecraft. Chapter 7 discusses the need to detect and limit nonliving contaminants of concern.

Finally, in Chapter 8, drawing on the information in Chapters 1 through 7, the committee presents its findings and recommendations. Chapter 9 concludes the report by providing a roadmap for the implementation of these recommendations.

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2

Policies and Practices in Planetary Protection

Planetary protection policy stems from international treaty, but it is implemented through practices within the national agencies that deploy probes to Mars. Planetary protection policies and practices are not static; they change considerably over time to reflect advances in scientific knowledge, new technologies, and the practical experiences of space agencies that launch planetary missions. This chapter discusses the history of planetary protection policies as promulgated by COSPAR and implemented in the U.S. space program. It explains the central concepts that link planetary protection policies, mission requirements, and standard practices, and it shows how COSPAR policies are translated into detailed processes of spacecraft preparation intended to prevent the forward contamination of Mars. This chapter also highlights how advances in science and technology have contributed to modifications in all three areas: the policies themselves; the specific requirements imposed by agencies to implement these policies; and the accepted practices or methods used by mission personnel to meet particular requirements.

PLANETARY PROTECTION POLICY

Historical Review

During the early years of the space program, forward contamination controls for missions to Mars were guided by a probabilistic approach as the framework for developing quarantine standards.[1] COSPAR Resolution No. 26, which COSPAR issued at its 1964 Scientific Assembly in Florence, Italy, accepted "a sterilization level such that the probability of a single viable organism aboard any spacecraft intended for planetary landing or atmospheric penetration would be less than 1×10^{-4}, and a probability limit for accidental planetary impact by unsterilized flyby or orbiting spacecraft of 3×10^{-5} or less" (COSPAR, 1964, p. 26). In 1969 COSPAR agreed to "a probability of no more than 1×10^{-3} that a planet will be contaminated during the period of biological exploration" and adopted a formal probabilistic approach focused on the probability of contamination, P_c (COSPAR, 1969, p. 15).

[1]For the early development of the probabilistic approach to planetary protection, see Sagan and Coleman (1965, 1966). Early thinking about planetary protection may be traced in two reports by the COSPAR Committee on Contamination by Extraterrestrial Exploration (CETEX, 1958, 1959), and in studies by Lederberg and Cowie (1958), Lederberg (1960), Brown et al. (1962), Atwood (1966), Hall (1968, 1971), and DeVincenzi et al. (1998), and references therein.

The 1964 COSPAR policy also incorporated the notion that "all practical steps should be taken to ensure that Mars be not biologically contaminated until such time as this search [for extraterrestrial life] can have been satisfactorily carried out . . ." (COSPAR, 1964, p. 26). To that end, COSPAR called for limiting the probability of an accidental planetary impact by unsterilized spacecraft as well as reducing spacecraft microbial bioburdens to specified levels. NASA policy incorporated the requirement that "outbound automated spacecraft and planetary exploration programs shall not, within [established] probabilities . . . transport terrestrial life to planets until it is determined that life does or does not exist on the planet and the character of existing life is explored" (NASA, 1967, p. 1). A finite period of time into the future by which the search for extraterrestrial life on Mars would be completed became known as the "period of biological exploration" (COSPAR, 1969).

During the 1960s and 1970s, the period of biological exploration was described in two different ways: (1) it was estimated as the time span it would take either to send a certain number of spacecraft to, or conduct a certain number of experiments on, Mars (Sagan and Coleman, 1965, 1966) and (2) it was translated into an absolute number of years, for example, the 20-year period from 1968 to 1988 (COSPAR, 1969).[2] According to Hall (1968), studies at that time indicated that accidental impacts of spacecraft on the martian surface and premature entry of orbiting vehicles into the martian atmosphere represented principal forward contamination concerns. Concerns about non-nominal (accidental) impact and the requirement for orbital spacecraft to achieve on-orbit lifetimes of at least 50 years are still reflected in planetary protection policies today, although time spans are rolling time limits now that are reset for each mission.[3]

Probability of Contamination and Probability of Growth

Historically, the approach used in establishing planetary protection requirements for spacecraft sent to Mars was to require that the probability of contamination (P_c) with terrestrial microorganisms—that is, the probability that Earth microorganisms introduced to Mars would then reproduce in situ on Mars—be below some threshold. One approach was to require that P_c multiplied by the total number of missions expected to be sent to Mars during the period of biological exploration would remain small compared with 1, that is, that the probability of contamination summed over all missions would remain small. Thus, P_c was set to 10^{-3} for all spacefaring nations, with different nations then being allotted fractions of this probability (COSPAR, 1969).

This approach depends on efforts to estimate P_g, the probability that Earth microorganisms will grow in the martian environment. The probability of contamination can be written as

$$P_c = N_0 R P_S P_I P_R P_g \tag{2.1}$$

where N_0 is the total number of organisms present on the spacecraft prior to bioburden reduction steps, R is the bioburden reduction factor achieved by any pre-launch sterilization procedures, P_S is the probability of surviving exposure to radiation, vacuum, temperature fluctuations, and so on during spaceflight and entry onto the planet's surface, P_I is the probability of impact on the surface (of interest for flybys or orbiters intended to avoid the surface, or for landers that risk non-nominal impacts with the surface), and P_R is the probability of release of microbes into the environment.[4] As written, Equation 2.1 assumes that P_c is small compared to unity.[5] Therefore,

[2]For example, "60 landers and 30 flyby and orbiter missions and a total of 1200 biological experiments on Mars" (Sagan and Coleman, 1965). Also, "the period of unmanned martian exploration shall be assumed not to extend beyond the year 2000, followed by manned exploration" and "the years 1966 to 2000 shall be considered the period for unmanned exploration . . . a total of 64 interplanetary flights toward Mars are expected" (Light et al., 1967).

[3]That is, 20- and 50-year time periods of relevance to planetary protection requirements set for each mission upon mission launch; see NPR 8020.12C (NASA, 2005a), p. 63. Available at <planetaryprotection.nasa.gov/pp/index.htm>.

[4]The particular form of Equation 2.1 varies from study to study, depending on which factors are collected into a single variable or broken out for individual assessment (e.g., P_S, the probability of surviving spaceflight, is sometimes written as a product of P_{VT}, the probability of surviving exposure to space vacuum and temperature, and P_{UV}, the probability of surviving exposure to ultraviolet light, during the voyage). For alternate conventions in writing Equation 2.1 see, for example, Klein (1991) and NRC (1978, 1992). The formulation given here is perhaps closest to that used by Stabekis, as described in P.D. Stabekis, Lessons learned from Viking, presentation to the Committee on Preventing the Forward Contamination of Mars, February 27, 2004.

[5]For an early exact formulation, see Sagan and Coleman (1965, 1966).

once P_c is fixed by policy, a given estimate of P_g then places a limit that the intervening product of factors must meet for that P_c to be achieved. In particular, estimates of P_g drive the requirements for bioburden reduction R.

Current NASA practices for estimating bioburden (be it N_0 or N_0R, the bioburden present after bioburden reduction measures) on spacecraft are by proxy (as measured by colony-forming units after heat shock at 80°C followed by incubation for 72 h; see Appendix C). This procedure has served as a cornerstone for estimating microbial burden on spacecraft and will continue to do so in the near term. However, recent advances in microbial ecology reveal two significant limitations to these spore-based estimates of numbers of bacteria on the spacecraft. As detailed in Chapter 5, molecular diversity surveys demonstrate that cultivation techniques fail to recover as many as 99 percent of the microbes in a microbial population (Pace, 1997). By logical extension, the technologies on which current NASA estimates of bioburden are based will not detect the majority of heat-resistant organisms on the spacecraft. More important, spore-based estimates tell little about the cellular physiology or genomic diversity of organisms on the spacecraft surface or within enclosed components, both of which directly influence P_g and provide valuable new information on possible strategies for reducing bioburden (see Chapter 6). The heat treatment protocol means that those colony-forming units that are found are likely due to spore-forming organisms capable of surviving heat shock. However, this spore proxy can record only what grows within a few days under a given set of laboratory conditions; it does not consider what might grow under different environmental conditions or over protracted periods. That is, it does not survey many organisms that may occur in the clean-room environment but about which little is known. The use of proxies in estimating spacecraft contamination therefore brings with it inherent risks.

Reasonably robust models could estimate levels of bioburden reduction during flight (P_S) by considering the presence of radiation-tolerant and heat-tolerant microorganisms, if these data were available. Estimates of P_I may be derived from actual crash data, and engineering models may assign values to P_R. In contrast, there is little scientific basis for estimates of P_g, and estimates of P_g have in fact varied by as much as 10 orders of magnitude during the period from 1964 to 1978 (Klein, 1991) (see Appendix D).

The Viking program, consisting of two orbiters and two landers that were launched in the mid-1970s, provided for the first time significant in situ scientific data on the martian environment. Following analysis of the Viking results, NASA asked the National Research Council (NRC) to evaluate P_g comprehensively, based on available knowledge of planetary conditions and the limits of life known at the time. The NRC report *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan* (1978) addressed planetary protection policy for exploratory missions to solar system locations that had been launched or planned for launch between 1974 and 1994. In that report P_c was stipulated as less than 1×10^{-3} for each planet, and P_g values were set separately for three different regions on Mars based on a "comparison between the known physical and chemical limits to terrestrial growth and the known and inferred conditions [on Mars]" (NRC, 1978, p. 4). The P_g values for Mars were set at $<10^{-10}$, 10^{-8}, and 10^{-7} for above- and below-surface subpolar areas and the polar caps, respectively. Although the report stipulated quantitative values for P_g, the values were arrived at subjectively and became a matter for debate.[6] In fact, the 1992 NRC report noted, "It is clear that considerable uncertainty has been engendered by the probabilistic approach to planetary protection. This concern has been restated over the years by virtually every group that has analyzed the problem, and indeed by NASA" (NRC, 1992, p. 44). The debate over the probabilistic approach to planetary protection continued in the years following the 1978 report and set the stage for a subsequent significant overhaul of planetary protection policy.[7]

[6]As noted in NRC 1992: "Although the [1978] committee expressed a reluctance in recommending a particular value for P_g, they argued that while the P_g for Mars is exceedingly low, the probability is not zero" (p. 44). The 1978 report had stated: "And yet a numerical value for P_g is required in order to determine what procedures are needed to reduce the microbial burden on future spacecraft to Mars to levels that fulfill current COSPAR quarantine policy. *Reluctantly, then, we recommend for these purposes, and these purposes alone, that NASA adopt a value of P_g less than 10^{-10} for the subpolar region . . .*" (italics in original).

[7]No Mars missions were ever flown whose forward contamination polices were based on the recommendations of the 1978 NRC report.

Mission Categories

After release of the 1978 NRC report, NASA undertook studies aimed at simplifying planetary protection procedures for upcoming missions and exploring ways to minimize the use of mathematical models and quantitative analyses. In 1982, NASA submitted a report to COSPAR suggesting that the previous quantitative policy be replaced by an entirely new approach (DeVincenzi et al., 1983). In 1984, COSPAR formally accepted the proposed approach and adopted a new policy centered on target-mission categorizations (COSPAR, 1984), an approach that has served since as the framework for planetary protection policy. This approach has also accommodated revisions to policy based on new scientific information about Mars obtained since 1984. Table 2.1 summarizes current COSPAR mission categories.

Although the probabilistic quantitative approach to determining P_c was eliminated in 1984 as the central concept in COSPAR policy, current Category IV and V missions still require a contamination analysis plan that focuses on many of the same variables that were the focus of earlier planetary protection policies (e.g., probability of impact, orbital lifetimes, microbial densities and bioburden, time-temperature sterilization requirements, and so on).[8] These are summarized in the section below titled "Implementation Requirements."

NASA adopted the revised COSPAR policy for application to all solar system exploration missions beginning with the Galileo mission in 1989. In 1990, in anticipation of upcoming robotic missions to Mars, NASA requested that the NRC revisit the matter of the potential forward contamination of Mars and provide recommendations that could become the basis for updating the requirements for forward contamination controls for Mars landers. The NRC report *Biological Contamination of Mars: Issues and Recommendations* (NRC, 1992) endorsed the use of categories rather than probability values as a "significant step forward in the process of simplifying and implementing planetary protection procedures" (NRC, 1992, p. 45), noting that "it is difficult, if not impossible" to estimate the potential for biological contamination of Mars (NRC, 1992, p. 48). The report also asserted that since "the P_g value for terrestrial organisms on Mars is so small as to be of no consequence . . . the need for severe reduction of spacecraft bioload solely to prevent the spread of replicating terrestrial organisms on Mars is no longer paramount" (NRC, 1992, p. 46). However, the report emphasized that the reduction of bioburden on all lander missions to Mars must continue to be addressed out of concern that life-detection measurements on future missions could be jeopardized through contamination of Mars by previous missions.

The report recommended a revised approach for planetary protection controls whose stringency was based on whether or not a mission carried instruments for in situ life-detection experiments. Those recommendations for planetary protection controls made explicit reference to the levels of cleanliness used in the Viking missions of the mid-1970s. Thus, biological contamination levels for spacecraft without life-detection experiments would be "subject to at least Viking-level pre-sterilization procedures—such as clean-room assembly and cleaning of all components—for reduction of bioburden, but such spacecraft need not be sterilized" (NRC, 1992, p. 47). In contrast, missions "carrying instrumentation for in situ investigation of extant martian life [were] subject to at least Viking-level sterilization procedures."[9] (Requirements for meeting Viking pre-sterilization and post-sterilization bioburden reduction levels are summarized in Table 2.2.) This approach freed planetary protection requirements from any explicit reliance on P_g, although it should be remembered that the policy requirement that P_c lie below a certain value, coupled with a choice (e.g., Viking levels) for R makes an implicit assumption, described by Equation 2.1, about the value of P_g that is valid for Mars.

COSPAR approved the recommendations proposed in the 1992 NRC report and refined the planetary protection policy for Mars missions to allow for different requirements on missions with and without life-detection

[8]In current contamination analysis plans, some numerical values of important parameters and specifications are assigned by the NASA planetary protection officer at initiation of the project rather than calculated as they were long ago. For example, based on the current policy for robotic extraterrestrial missions, the period of biological exploration is interpreted as 50 years and is reflected in the probability-of-impact values and assigned orbital lifetimes currently used. Specifically, "Orbit characteristics shall be such that P_I max for a mission shall be met until 20 years from launch of the mission. Between 20 and 50 years from launch, the spacecraft shall remain in orbit with an assurance of ≥0.95" (NPR 8020.12C; NASA, 2005a). Documents such as NPR 8020.12C are available at <planetaryprotection.nasa.gov/pp/index.htm>.

[9]Viking post-sterilization bioloads begin with $N = 3 \times 10^5$ spores allowed (Viking pre-sterilization level) and apply a sterilization process to reduce the total N by 4 orders of magnitude—equivalent to 30 surface spores. See Table 2.2.

TABLE 2.1 COSPAR Planetary Protection Policy Categories for Solar System Bodies and Types of Missions (and Specific Requirements for Mars)

	Category			
	I	II	III	IV
Mission type	Any but Earth return (flyby, orbiter, lander)	Any but Earth return (flyby, orbiter, lander)	No direct contact (flyby, some orbiters)	Direct contact (lander, probe, some orbiters)
Target body	Venus; Moon; undifferentiated, metamorphosed asteroids; others TBD	Comets, carbonaceous chrondite asteroids, Jupiter, Saturn, Uranus, Neptune, Pluto/Charon, Kuiper Belt objects, others TBD	Mars, Europa, others TBD	Mars, Europa, others TBD
Degree of concern	None	Record of planned impact probability and contamination control measures	Limit on impact probability Passive bioload control	Limit on probability of non-nominal impact Limit on bioload (active control)
Representative range of requirements	None	Documentation only: planetary protection plan, pre-launch report, post-launch report, post-encounter report, end-of-mission report	Documentation (Category II) plus contamination control, organics inventory (as necessary); implementing procedures such as trajectory biasing, clean room, bioload reduction (as necessary) Mars orbiters will not be required to meet orbital lifetime requirements[b] if they achieve bioburden levels equivalent to the Viking lander pre-sterilization total bioburden.	Documentation (Category II) plus P_c analysis plan, microbial reduction plan, microbial assay plan, organics inventory; implementing procedures such as trajectory biasing, clean room, bioload reduction, partial sterilization of contacting hardware (as necessary), bioshield monitoring of bioload via bioassay

[a]A special region is defined as a region within which terrestrial organisms are likely to propagate, or a region that is interpreted to have a high potential for the existence of extant martian life forms. Given current understanding, this definition applies to regions where liquid water is present or may occur. Specific examples include but are not limited to (1) subsurface access in an area and to a depth where the presence of liquid water is probable, (2) penetrations into the polar caps, and (3) areas of hydrothermal activity.

IVa	IVb	IVc	V
			Earth return
Mars	Mars	Mars	Mars, Europa, others TBD
			If restricted Earth return: no impact on Earth or the Moon, returned hardware sterile, containment of any sample *Outbound* Same category as target body/outbound mission *Inbound* If restricted Earth return: Documentation (Category II) plus P_c analysis plan, microbial reduction plan, microbial assay plan, trajectory biasing, sterile or contained returned hardware, continual monitoring of project activities, project advanced studies/research If unrestricted Earth return: none
Lander systems not carrying instruments for the investigations of extant martian life are restricted to a biological burden no greater than Viking lander pre-sterilization levels.	For lander systems designed to investigate extant martian life, all of the requirements of Category IVa apply, along with the following requirement: The entire landed system must be sterilized at least to Viking post-sterilization biological burden levels, or to levels of biological burden reduction driven by the nature and sensitivity of the particular life-detection experiments, whichever are more stringent, *or* the subsystems that are involved in the acquisition, delivery, and analysis of samples used for life detection must be sterilized to these levels, and a method of preventing recontamination of the sterilized subsystems and the contamination of the material to be analyzed is in place.	For missions that investigate martian special regions,[a] even if they do not include life-detection experiments, all of the requirements of Category IVa apply, along with the following requirement: Case 1: If the landing site is within the special region, the entire landed system shall be sterilized at least to the Viking post-sterilization biological burden levels. Case 2: If the special region is accessed through horizontal or vertical mobility, either the entire landed system shall be sterilized to the Viking post-sterilization biological burden levels, *or* the subsystems that directly contact the special region shall be sterilized to these levels, and a method of preventing their recontamination before accessing the special region shall be provided.	

[b]Defined as 20 years after launch at greater than or equal to 99 percent probability, and 50 years after launch at greater than or equal to 95 percent probability.

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TABLE 2.2 Current Bioburden Requirements for Mars Landers (Viking Pre- and Post-Sterilization Levels for Surface and Embedded Bioburden)

	IVa: Viking		IVb: Viking
	Pre-Sterilization	Pre-Sterilization with Impacting Hardware	Post-Sterilization
Surface spore density	300 spores/m^2	300 spores/m^2	No explicit requirement
Total surface spores	3×10^5	3×10^5	30[a]
Total spores (including surface, mated, and embedded)	__[b]	5×10^5	__[b]

NOTE: Embedded or encapsulated bioburden refers to bioburden buried inside nonmetallic spacecraft material.
[a]No surface spore assays required; number of spores established by the application of a certified bioburden reduction method (dry heat).
[b]No numerical requirements on embedded bioburden except for spacecraft with impacting hardware (e.g., heat shields). Embedded bioburden is assumed to remain embedded under nominal operations.
SOURCES: Perry Stabekis, The Windermere Group, and Jack Barengoltz, Jet Propulsion Laboratory, personal communication, January 2005; NPR 8020.12C (NASA, 2005a).

instruments (Categories IVa and IVb) (DeVincenzi et al., 1994). In 2002, due to concerns about ensuring adequate control of forward contamination during exploration, COSPAR again modified the planetary protection policy by adding a new category, Category IVc, for missions that investigate martian "special regions," even if those missions do not include life-detection experiments. A special region was defined as "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant Martian life forms" (COSPAR, 2003, p. 71).[10] The changes made at that time in COSPAR policy stipulated additional bioburden reduction and sterilization requirements for landers or components that would come into contact with special regions or have a high probability of involving other than nominal conditions (see Table 2.1).

Current planetary protection practices for U.S. missions to Mars incorporate all the revisions to COSPAR policy through 2002.[11] NASA continues to work actively with COSPAR and the international community in considering whether and how COSPAR policies and associated implementing regulations should be revised to reflect rapidly changing understanding of both Mars and microbial life.

IMPLEMENTATION REQUIREMENTS

Historically, the various NRC recommendations on modifications to planetary protection policies (see Appendix B) have been adopted for use in revisions to COSPAR's policies. In practical terms, they have been translated into forward contamination controls for NASA missions in the form of procedural requirements for actions under the control of mission designers, spacecraft and equipment builders, and planetary protection technicians. The current overall planetary protection policy is specified in NASA Policy Directive (NPD) 8020.7F (NASA, 1999), which applies to both robotic and human missions.[12] NASA Procedural Requirements (NPR) document 8020.12C

[10]The text added, "Given current understanding, this is to apply to regions where liquid water is present or may occur. Specific examples include, but are not limited to: Subsurface access in an area and to a depth where the presence of liquid water is probable; Penetrations into the polar caps; [and] Areas of hydrothermal activity."

[11]The formal incorporation of Category IVc (missions to special regions) into NASA policy received final administrative approval in NASA NPR 8020.12C in 2005.

[12]NPD 8020.7F provides details on NASA's policy on "Biological Contamination Control for Outbound and Inbound Planetary Spacecraft." Although current implementing regulations apply only to robotic spacecraft, the NPD specifically applies to human spaceflight as well: NASA "will ensure that applicable standards and procedures established under this policy, and detailed in subordinate implementing documents, are incorporated into human missions."

(NASA, 2005a) and NPR 5340.1 (NASA, 1980)[13] together describe the various elements in NASA's planetary protection implementation requirements, as well as the standard methods used to implement those controls.

Planetary Protection Plan

Currently, missions to Mars fall into one of two COSPAR categories—Category III for orbiters and flybys, and Category IV for "direct contact" missions using, for example, landers, penetrators, and airplanes. Both Category III and IV missions require the development and approval of a planetary protection plan that provides information on all aspects of the mission, from pre-launch preparations through end-of-mission reports. NPR 8020.12C (NASA, 2005a) stipulates that for a flight project to demonstrate compliance with NASA planetary protection requirements, the mission team must develop a planetary protection plan and obtain approval of the plan from the NASA planetary protection officer. Based on the category assigned by NASA to a particular mission, different implementation guidelines and specific requirements outlined in the NPR apply to trajectory biasing, clean-room assembly, microbial reduction and assaying, organics inventory and archiving, and recontamination control. In addition, the NPR establishes requirements for documentation and schedules for reviews, and it provides assigned quantitative values for specifications on a wide variety of parameters.[14] Specific information required in mission plans includes analysis of the probability of impact, estimates of microbial bioburden, a contamination analysis plan, microbiological assay plans, a microbial reduction plan if contemplated, and reporting plans and schedules. In addition, Category IV landers and probes must collect and archive an organics inventory, and document measures for avoidance of recontamination (e.g., bioshield monitoring) before launch. The requirements and plans that can have the most significant implications for the design, development, assembly, and cost of the mission are typically those related to reducing and assessing the bioburden on the spacecraft.

The requirements for missions to Mars fall into the following important areas: (1) reduction of biological contamination from various sources on the spacecraft hardware; (2) consideration of non-nominal impact avoidance; (3) use of required assay methods for verifying bioburden reduction on the spacecraft and maintenance of clean-room conditions; (4) documentation to show that a spacecraft subjected to cleaning and bioburden reduction has not been recontaminated up until launch time; (5) development and maintenance of inventories of bioburden and organic constituents of the spacecraft and its components; and (6) planning and scheduling of required documentation extending from pre- and post-launch plans through end of mission reports. Four of those six areas—microbial reduction and bioburden control, use of standard assay methods, development and maintenance of archived inventories, and impact avoidance—have particularly important implications for the current implementation of forward contamination controls for missions to Mars. Table 2.3 provides descriptions of all six areas.

Bioburden Reduction

Bioburden or microbial reduction[15] is of great importance because it significantly affects the densities of microbial contaminants on a spacecraft and/or its component parts before launch, thereby reducing the potential for forward contamination of a planet. Bioburden reduction may be accomplished in a variety of ways—performed on either the entire spacecraft or its component parts and pieces. Depending on the COSPAR category assigned to

[13]NASA's NPR 8020.12C (NASA, 2005a) provides the detailed planetary protection provisions for robotic extraterrestrial missions. NPR 5340.1 (NASA, 1980) is currently being revised and provides standard procedures for the microbial examination of space hardware and associated clean-room assembly and pre-launch environments. NPR 5340.1C (NASA, 2005b) is an informal reissue of NHB 5340.1B. A revised NPR 5340.1D is pending formal approval at the time of this writing.

[14]Various pertinent parameters and specifications are used to address how contamination controls will be implemented on a particular mission. Appendix B of NPR 8020.12C (NASA, 2005a) provides quantitative values and acceptable ranges for specifications related to clean-room requirements, probability of accidental impacts, material-related microbial densities, microbial burden assays, sterilization time-temperature specifications, and planet-specific requirements (e.g., orbital lifetimes).

[15]The term "microbial reduction," which has the same meaning as bioburden reduction, is used in this chapter to be consistent with the terminology used in the NASA requirements documents discussed throughout the chapter. Other sections of this report use the term "bioburden reduction."

TABLE 2.3 Elements of Planetary Protection Forward Contamination Controls

Planetary Protection Elements	Requirements, Parameters/ Methods[a]	Standard Methods[b]	Comments
Bioburden Control			
1. Facility: clean-room requirements	X[c]	X	Contamination control, microbial culture assays, and monitoring must be demonstratebly effective; facility certification; current assay methods based on culture growth for 72 h under varied conditions
2. Hardware decontamination methods			
2a. Dry-heat/sterilization procedure (preferred method)	X[c]		Time-temperature conditions and *D*-values[d] specified for bioburden of exposed, mated, and encapsulated materials
2b. Alternative methods for hardware decontamination	X[e]		Must demonstrate effectiveness in reducing bioburden; approval by the planetary protection officer; no standard certification process exists for new methods
Non-Nominal Impact Avoidance	X		Total probability of any accidental impact by hardware other than probe or lander modules must not exceed 10^{-4}
Assay Methods			
1. Standard assay methods to determine hardware microbial burden		X	Methods based on culturing and colony growth for 72 h under varied specified conditions
2. Alternative procedures for assaying	X[e]		May be proposed, but no standard certification process exists for new methods
Protection from Recontamination			
Assaying and monitoring Use of microbial barriers Macro-organism control Contingency planning	X		Specifications for shrouds, filters, seals, and so on, are provided in the requirements document. Assay methods same as standard assay methods (1) above
Bulk Organics Inventory			
a. Parts and materials lists b. Samples of organic compounds c. Location of landing and impact points d. Condition of landed spacecraft (to track spread of organics)	X		Archiving planetary-protection-related information: Flight program office must provide for collection and storage of information for at least 20 years from the launch of the spacecraft. No requirement currently exists for the collection and storage of microbial assay information
Documentation Required			
Planetary protection plan/requirements compliance Schedules Pre-launch planetary protection report Post-launch planetary protection report End-of-mission report	X		Administrative requirements of Planetary Protection Office

[a]Stipulated in NPR 8020.12C (NASA, 2005a).
[b]Described in NPR 5430.1C (NASA, 2005b).
[c]Appendix A of NPR 8020.12C.
[d]A *D*-value in the context of spacecraft sterilization is the time required at a specific temperature to cause a 1 log decrease in the spore population.
[e]Allowed, but not specified.

a mission, the mission team is tasked with ensuring that spacecraft bioburden does not exceed the levels specified for that category during the entire process encompassing assembly, test, and launch operations (ATLO). As outlined in NASA requirements (NPR 8020.12C; NASA, 2005a), microbial reduction for an entire planetary spacecraft, including entry probes and landing capsules, may be accomplished by any qualified process approved by the planetary protection officer.

At present, the dry-heat cycle is considered the preferred method for conditioning spacecraft to a sterile or near-sterile condition, and it is the only NASA-certified method for bioburden reduction.[16] A dry-heat cycle involves heating the entire spacecraft or particular components to specified elevated temperatures and atmospheric conditions for defined lengths of time. Mission managers may use alternative methods of microbial reduction provided that no undue reduction of hardware reliability occurs and the method is supported by rigorous data demonstrating biological effectiveness and reproducibility.

Because many components of modern spacecraft are particularly sensitive to heat (e.g., electronics, some nonmetallic portions), it is often desirable to reduce the severity of the subsequent heat sterilization by precleaning individual parts and components. In those cases, certain elements of hardware are subjected to separate microbial reduction processes prior to their assembly into the entire spacecraft. The microbial reduction methods used on such hardware components and pieces may differ from those used for the entire spacecraft. In all cases, however, the methods themselves must be preapproved to ensure biological qualification, quality assurance of the method, and demonstration of nondegradation of parts to ensure that they are able to withstand any subsequent microbial reduction performed on the entire spacecraft. Although alternative methods may be approved for use on hardware components, there is no standard process for certifying new methods as qualified bioburden reduction procedures. Chapter 6 details several alternatives to heat sterilization, including chemical and radiative techniques.

According to NASA requirements (NPR 8020.12C; NASA, 2005a), calculations of the microbial reduction process must be supported by detailed information on the methods used throughout the cleaning processes (so-called parameter values and specifications), as well as data from reproducible laboratory tests or technical references supplied by the project team. Important parameters in the calculations include factors such as clean-room conditions, encapsulated and surface microbial density, sterilization cycle times, temperature constraints, process atmospheric conditions, *D*-values[17] for various bioburden categories (exposed, mated, and encapsulated), minimum number of spores per assay, and survival of hardy organisms after nominal cycles. Appendix E outlines the approaches taken to bioburden reduction on several past lander missions to Mars.

Standard Microbiological Assays for Assessing Microbial Contamination Levels

NASA's NPR 5340.1 (NASA, 1980) defines the accepted standard procedures for assessing the amount of microbial burden on space hardware and in associated ATLO facility environments. Specifically, it describes "uniform microbiological assay procedures that shall be used to: (a) Assess the degree of microbiological contamination of intramural environments where spacecraft hardware is assembled, tested and launched [and] (b) Assess the level of microbiological contamination of spacecraft hardware in relation to the known or anticipated environments of the target planets" (NASA, 1980, p. 3).

In addition, NPR 5340.1 (NASA, 1980) provides protocols for preparation and sterilization of both equipment and culture media used in association with the assay methods. Appendix C of this report summarizes methods for

[16]Other acceptable and approved methods (e.g., wiping with alcohol) are also used to reduce microbial bioburden on surfaces; however, these other processes are not certified and must be followed by standard assay methods to document the cleanliness levels achieved. A "certified process" is one for which a "credit for microbial reduction" is granted by virtue of using the certified process alone, with no further assaying required. Dry-heat microbial reduction is the only *certified* microbial reduction process; its efficacy has been fully documented. Because the dry-heat process is associated with strict specifications and assigned parameter values, microbial reduction is assumed and no further assaying is required.

[17]The *D*-value for bioburden on exposed surfaces is defined as the "time required to destroy 90% of the microbial spore population on surfaces subjected to sterilizing dry heat at a temperature of 125°C at an absolute humidity corresponding to a relative humidity of less than 25% referenced to the standard conditions of 0°C and 760 torr pressure" (NPR 8020.12C; NASA, 2005a).

each type of assay for the various categories of material and locations assayed (e.g., clean-room air and surfaces, spacecraft hardware by size categories, and so on). In all cases, suitable controls are also specified for the assay processes and procedures. Assays play two important roles before the launch of a spacecraft: (1) they allow monitoring by flight program or project personnel for microbial contamination throughout the ATLO process (if surface contamination is detected, particular parts are removed and recleaned and then replaced on the spacecraft); and (2) they certify the required cleanliness levels of environments, facilities, and flight hardware for the planetary protection officer at particular phases of the ATLO process. Assays are often performed by a technical organization designated by the planetry protection officer.

In accounting for the bioburden of pre-launch spacecraft, attention is focused mainly on two types of microbial burden—surface and encapsulated (embedded)[18]—based on the location on or in the spacecraft. Surface bioburdens are actually measured through standard microbial assays, whereas values for encapsulated microbial densities inside nonmetallic materials or portions of the spacecraft are assigned as parameter values (NPR 8020.12C; NASA, 2005a). Because embedded or encapsulated bioburden cannot be accessed or measured, there is currently no quantitative indication of its phylogenetic diversity or the densities of the various types of microbes. In addition, the assigned parameter values are based on pre-Viking data,[19] and so they may not reflect values appropriate for current (4 decades later) spacecraft materials or manufacturing processes.

Archived Information and Organics Inventories

The primary assay data for each mission are compiled and submitted to the planetary protection officer as part of the planetary protection plan for each mission, providing verification of a mission's having met planetary protection requirements. For all current and past missions, such data are obtained from standard swab and culture assays, rather than from more sensitive modern molecular methods. This means that although there are archival data on levels of contaminants from previous outbound missions, they provide almost no information on the phylogenetic diversity or actual density of the individual types of microbial bioburden. Using contemporary molecular techniques, microbiologists have successfully cultivated between 0.1 and 1 percent of the different kinds of organisms from complex microbial communities. Historically, microbial cultures have not been required to be retained routinely. Today, despite the fact that planetary protection controls are being implemented on all missions whose categorizations require it, the controls do not require a comprehensive understanding of the actual bioburden levels or of the phylogenies of the microbes on the spacecraft. Thus, a comprehensive understanding of potential forward contamination of Mars remains elusive.

Finally, in attending to the forward contamination control requirements for Category IV landers, the flight program office must provide for collection and storage of the bulk (>1 kg) organic constituents of all launched hardware that is intended to directly contact Mars or might accidentally do so. Parts and materials lists, actual samples, and information on landing and impact points must be maintained for at least 20 years after spacecraft launch. Given recent scientific findings about Mars and the fact that the period of biological exploration continues to be extended, this requirement may be insufficient for archiving scientifically important information.

Impact Avoidance

Although the meaning of the "period of biological exploration" has changed over time, the term remains in current policies and implementation requirements. In particular, Category III Mars spacecraft (flybys and orbiters) have to be able to guarantee orbital lifetimes of 20 and 50 years with probabilities of impacting Mars over those time periods of less than 1 and 5 percent, respectively. If the orbiter cannot meet those requirements, it has to meet

[18]Mated bioburden is also mentioned in planetary protection requirements, but quantitative standards are stipulated only for surface and encapsulated and embedded bioburden.

[19]See NPR 8020.12C (NASA, 2005a), pp. 39-41. Standards for average encapsulated microbial density are referenced to the Planetary Quarantine Advisory Panel Review, September 28, 1971, Denver, Colo.

the Category III bioburden requirement; that is, it must have its bioburden reduced to below 5×10^5 total aerobic spores (COSPAR, 2003; also see Table 2.1). But if the orbiter can meet these requirements—that is, if the probability is high that its impact will occur subsequent to the implicit period of biological exploration—then no special bioburden reductions are required beyond standard assembly in a class-100,000 clean room, and so forth (see Table 2.2).

MAINTAINING CLEANLINESS DURING LAUNCH

Preventing contamination of a Mars-bound spacecraft does not end with spacecraft assembly and bioburden reduction. Payloads, once cleaned, are kept in clean-room facilities that can maintain the level of cleanliness required for the spacecraft. If higher sterilization levels are achieved than can be ensured in the clean-room facilities, the payloads are put in a biobarrier to prevent recontamination. The biobarriers are not removed until the flight system is launched into space.

For U.S. Mars-bound spacecraft, the payload fairing[20] (otherwise referred to as the launch vehicle shroud) is installed in final-assembly clean rooms at Kennedy Space Center. Once the payload is within the payload fairing, it is under continuous conditioned air or gaseous nitrogen purge, which means that a positive pressure gradient within the fairing is maintained with the nitrogen exiting the fairing through high-efficiency particulate air (HEPA) filters. This state is maintained during the mating of the spacecraft to the launch vehicle and the movement of the mated payload out to the pad. If engineers require access through the payload fairing to reach the spacecraft while on the pad, the positive pressure is maintained, with the nitrogen flowing continuously out the access door. The nitrogen source is disconnected at the moment of launch, but outflow continues as the launch vehicle ascends, owing to the decrease in atmospheric pressure with altitude. The payload fairing is jettisonned at approximately 400,000 ft after launch, where little or no atmospheric contamination remains. The payload fairing separation mechanisms are designed and tested so that virtually no particle debris from the jettison operation will contact the spacecraft.

In summary, little or no recontamination of the spacecraft should occur during launch. As an additional precaution, starting with the launch of the Mars Science Laboratory (MSL), scheduled for 2009, payload fairing manufacturers will be required to adhere to planetary protection cleanliness requirements for all inside surfaces of the shroud. These measures are being required to minimize any spore migration within the shroud.

CURRENT LIMITATIONS OF STANDARD METHODS AND IMPLEMENTING REQUIREMENTS

Although COSPAR's planetary protection policies and NASA's implementation requirements have been modified over time, the standard NASA methods and practices for cleaning, sterilizing, and assaying spacecraft in preparation for launch have remained largely unchanged for nearly 3 decades. Even the 1992 NRC report on Mars forward contamination accepted the continuing use of established practices while encouraging the development and adoption of more modern molecular methods for assaying spacecraft and spacecraft assembly clean rooms (see Appendix B). For the most part, the standard methods and practices were developed through an extensive research program during the early years of the space program, particularly before the launch of the Viking landers (Bionetics, 1990). In addition, there is no standard certification process for approval of new methods, whether for microbial reduction or assaying, both of which currently require extensive documentation of process effectiveness, reproducibility, and equipment reliability before approval as alternate methods. There are no requirements for

[20]The payload fairing (also referred to as the launch vehicle shroud) is the uppermost section of the launch vehicle that encloses the payload, protecting it from atmospheric dynamic pressure and heating during the initial ascent through the atmosphere. The payload fairing is typically designed in two sections resembling a clamshell. At burnout of the first stage of the launch that occurs around 400,000 ft, the payload fairing is jettisoned with explosive bolts that separate the fairing at the clamshell seam and at its base, where it interfaces with the launch vehicle adapter. The two halves fall away from the launch vehicle before the second stage is ignited and the launch flight continued.

developing and maintaining information on microbial phylogenetic diversity and densities in clean-room environments or on prelaunch spacecraft. Moreover, the period required for retaining organic inventory archival information is relatively brief in relation to the lengthening period of biological exploration.

The committee has identified a number of limitations to the NASA standard methods and planetary protection requirements. In particular, improvements in implementation practices and/or additional research are needed in areas related to (1) bioburden reduction and sterilization methods; (2) assigned parameter values and specifications used in contamination control planning; (3) microbial assay methods based on quicker, more sensitive molecular techniques that yield greater resolution of actual microbial bioburdens; (4) assessment of embedded bioburdens in contemporary spacecraft hardware; (5) characterization and inventory of the phylogenetic diversity and true microbial densities on spacecraft and in clean rooms before launch; and (6) time requirements for maintaining organics inventory information and materials. These improvements are especially needed in light of ambitious plans for future Mars exploration, new knowledge about the martian environment relative to life, and the limits of life on Earth that are discussed in the following chapters.

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3

Future Mars Exploration: The Rolling Wave

A 20-year hiatus in Mars exploration followed the 1976 Viking spacecraft landings on Mars. But since the Mars Pathfinder mission in 1996, NASA has pursued a Mars exploration strategy that entails new missions at every Mars launch opportunity—every 26 months. These opportunities have included both orbiter and lander missions with complementary capabilities. Orbiter missions provide global datasets and context, as well as communications for "direct contact" missions such as rovers. Landed missions provide detailed in situ measurements that can be related to the global perspective that the orbital missions provide. Current and upcoming Mars missions are shown in Figure 3.1 and Table 3.1.

Future Mars missions can be expected to identify and study regions on the planet that contain evidence for habitable environments, especially those that indicate the past or current presence of liquid water (MSPSG, 2004). Evidence for water on Mars is widespread (see Chapter 4). The 2005 Mars Reconnaissance Orbiter (MRO) will provide a large volume of new remote sensing data, including data from extreme close-up photography, imaging spectroscopy, and sounding radar. The 2007 Phoenix Mars Scout mission will explore a high-latitude landing site where near-surface water ice may be accessible, and the 2009 Mars Science Laboratory (MSL) will conduct detailed mobile in situ investigations that will assess the past habitability of a promising field site. Additional Mars Scout missions[1] will be interspersed with program missions and can respond to emerging and focused scientific questions that arise over the course of NASA's Mars exploration strategy. Planning is under way to select Scout missions in 2011, 2016, and 2020 in response to Announcements of Opportunities soliciting concepts for Scout missions. In the intervening launch opportunity years (2013, 2016, and 2018), program-directed missions (missions developed by NASA) including sample return, an astrobiology field laboratory,[2] and a deep-drilling mission are under consideration (see Table 3.1).

Together, these planned missions represent an unprecedented systematic exploration of an alien world. Over time, missions will become increasingly ambitious in both technology and objectives at Mars. Adaptations in scientific goals and techniques, as well as in planetary protection policies and practices, will need to be evaluated and implemented in a flexible manner in the midst of this rolling wave of exploration.

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[1]Scout missions are principal investigator (PI)-led missions, which means that a PI whose proposal is selected for a mission is responsible to NASA for the management of the mission and its scientific success.

[2]The Mars Astrobiology Field Laboratory would be a mobile mission intended to conduct a robotic search for life and detailed analysis of geologic environments thought to be conducive to life.

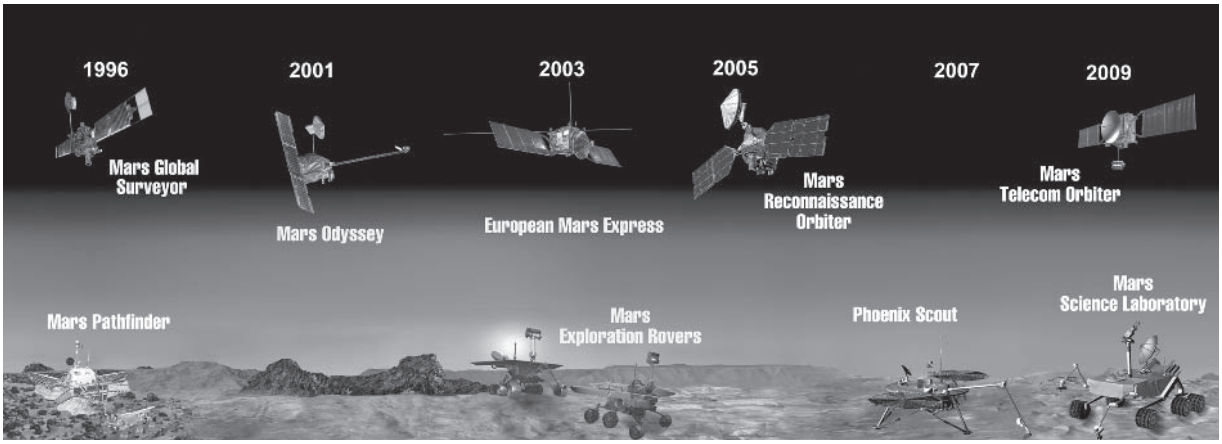

FIGURE 3.1 The suite of recently completed, current, and funded Mars missions planned for launch by the international community before the end of 2010. SOURCE: Presentation to the committee by James Garvin, then chief scientist of the Mars Program, NASA, February 26, 2004.

TABLE 3.1 Mars Exploration: Planned Missions and Year and Mission Concepts and Their Objectives

	Planned Missions and Year				Mission Concepts			
	2005	2007	2009	2011	2013	2016	2018	2020
Mission	MRO	NASA Phoenix Scout	MSL; Mars Telesat Orbiter	Mars Scout	Mars Sample Return *or* Astrobiology Field Laboratory	Mars Scout	Astrobiology Field Laboratory *or* Deep Drill; Network mission	Mars Scout; Deep Drill
Objective	Characterize the martian surface, subsurface, and atmosphere; identify potential sites for future landers	Explore the geologic history and biological potential of the northern plains of Mars	Investigate potential habitats for life	To be competitively selected	Collect fines, rock fragments, and atmosphere and return samples to Earth Explore site identified as habitat for life	To be competitively selected	Explore a site identified as a habitat for life Drill 3 to 10 m to gather samples Use global network of several landers to measure seismology and geochemistry	To be competitively selected

NOTE: This table represents the Mars Exploration Program as conveyed to the committee at the time of its study preparation. Since that time, the program has changed. The schedule for the Mars Sample Return mission has slipped several years, and the Mars Telesat mission has been canceled.
SOURCE: MSPSG (2004).

INCREASING COMPLEXITY, CAPABILITY, AND CREATIVITY

As the Mars Exploration Program unfolds, missions will take advantage of increasing complexity, capability, and creativity for exploring the planet. Future missions may rely on balloons, drills, and airplanes to tour and access the planet. For example, moles[3] and drills are techniques for enhancing access to the martian subsurface. The European Space Agency's Beagle II Lander was the first Mars mission that was to employ a mole; that device was designed to reach depths as great as 1.5 m beneath the surface.[4]

Because access to the subsurface is an important capability for conducting a number of high priority scientific and human exploration-related investigations (Beaty et al., 2001; Clifford et al., 2001; MEPAG, 2004), there is also considerable interest in, and active work on, the development of robotic drills capable of reaching depths of 2 to 100 m beneath the martian surface (Miller et al., 2004). Technologies capable of reaching depths of as much as several kilometers are also being considered (Blacic et al., 2000), motivated by the desire to reach any permanent reservoir of groundwater that might be present beneath the cryosphere (see Chapter 4). From an implementation perspective, the technical readiness level of current prototypes suggests that drilling investigations capable of reaching depths of up to ~25 m could be flown by 2020, while access to depths of 100 m or more may be possible by 2030.

Although access to depths of several kilometers may be decades away with the drilling technologies now envisioned, comparable depths may be reached in the permanent polar ice caps much sooner. For example, one concept to explore the polar subsurface incorporated a probe capable of melting its way through a few tens of meters of ice, with the resulting melt water freezing in its wake (Hecht and Saunders, 2003). Similar devices proposed for use in Earth's Antarctic regions and on Europa are theoretically capable of reaching depths of many kilometers.

Future missions to the martian surface are also planned to have greater longevity and mobility. The Mars rovers Spirit and Opportunity rely on battery and solar power sources that were designed for 3-month operation, though their lifetime has proven to be much longer under martian conditions than anticipated, and both rovers were functioning more than 1 year after their landings. The 2009 Mars Science Laboratory, a mission designed to explore potential habitats, will use a radioactive thermal generator as a power source, giving the rover the capability to roam distances of 20 km and to visit multiple sites that may include diverse martian environments.

Mission concepts have also been proposed for conducting regional Mars exploration, through airborne devices. Balloons have been proposed to traverse large areas, borne by seasonal winds for durations of days to weeks, using either solar heating (in a summer polar region) or helium gas to maintain flotation. Reconnaissance of hundreds to thousands of square kilometers seems possible, as does vertical profiling of atmospheric properties. Mars airplanes using chemical propulsion or simply gliding in the atmosphere have also been proposed with similar capabilities, trading off reduced coverage for control of the specific surface areas to be investigated.

NASA's strategy for exploring Mars is exciting. As planned, the Mars Exploration Program will search for and visit those regions that suggest the best case for harboring present or past life.[5] Advanced technologies could enable touring and traversing the planet over diverse environments, including dry regions, icy areas, or even sites with permanent liquid water, should they exist. Mechanisms for accessing the subsurface and collecting samples could involve contact with martian regolith at depths that may have been protected from the harshness of the Mars surface and environment. The potential success of these missions in identifying habitats, locating past life, or even finding extant life could call for repeat visits by later probes to verify findings and conduct further scientific investigations. In light of the current plan for Mars exploration, as detailed in the *Mars Exploration Strategy* (MSPSG, 2004), the need for planetary protection practices to prevent the forward contamination of Mars is critical. Indeed, NASA's solar system exploration roadmap identifies planetary protection technologies as important for the next decade of solar system exploration (NASA, 2003). A challenge, however, is how to integrate the new knowledge obtained from the ongoing series of Mars exploration missions into planetary protection policies and practices.

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[3]A mole is an autonomous device that is capable of entering the subsurface.

[4]See <www.beagle2.com/index.htm>. The Beagle II lander failed, however, and may have crashed.

[5]See MSPSG (2004), pp. 8-11.

THE ROLLING WAVE

The Mars Exploration Program's 26-month launch cycle is providing the planetary science community with a rolling wave of Mars missions, including broad international participation, that promises increasing levels of technological capability and scientific discovery at each new mission opportunity. This rolling-wave strategy allows for a key attribute: the ability to feed information and capabilities from each mission forward into subsequent Mars mission to be flown. Such new knowledge includes (1) scientific findings; (2) landing site characterizations; (3) improved atmospheric, surface, and subsurface models; (4) new flight-proven capabilities in both instrumentation and flight systems; and (5) lessons learned from previous mission development and operation activities.

This strategy was at work with the Mars Global Surveyor, Mars Odyssey, and Mars Express missions, which provided remote sensing data that identified a number of promising targets for detailed in situ surface exploration, including signs of aqueous sedimentary deposits—environments that on Earth are known to preserve evidence of past life—and surface and near-surface water ice deposits that may have melted during the recent past. Data from the Mars Global Surveyor orbiter was crucial to the selection of sites that Mars Exploration Rover (MER) missions are currently exploring.

With sequential Mars launch opportunities separated by as little as 26 months, feeding information forward into succeeding missions represents a considerable challenge. By the time information from one mission is collected and digested into knowledge, the next mission is typically already assembled and in final testing for launch. Hence, much of the knowledge acquired from a Mars mission may not strongly influence exploration activities until two or even three missions later, though program plans for the number of missions, schedule, and pace of exploration could change over time. However, some information, such as the location and physical properties of promising landing sites, can benefit future missions even after they have been launched. For instance, the Phoenix and Mars Science Laboratory missions are expected to finalize their landing sites after they are launched, in order to benefit from the remote sensing data to be provided by the Mars Reconnaissance Orbiter.

NASA's approach to accommodating the rolling wave and the resulting information latency (the delay between when information is gathered and when it begins to influence mission design) is to alternate mission types in successive opportunities—orbiters, then landers, then orbiters—because critical information is often specific to the type of mission (e.g., orbiter versus lander). Thus, the agency has roughly 3 years (assuming the flight time to Mars and a 6-month science mission before scientific results are obtained) to integrate the results obtained by a lander, for example, into the next lander mission. The process has facilitated NASA's ability to use the knowledge acquired from Mars missions for subsequent exploration.

Preliminary plans for the Mars program of the 2010-2020 decade suggest that international partners could play an increased role both in the scope and level of their involvement in Mars exploration activities, intensifying the rolling wave of missions on and data from Mars. The European Space Agency (ESA) is conducting advanced planning for the ExoMars mission in the 2009-2011 time frame. It will include an orbiter as well as a lander/rover with the Pasteur payload designed to detect signs of past or present life. ESA has also announced the Aurora program,[6] which includes plans for Mars remote sensing, in situ exploration, a sample return mission, and eventually a robotic outpost and human exploration. Therefore, the specific mission set that NASA eventually flies will be affected by results from previous missions as well as evolving international plans. It may also be modified or augmented by NASA's Moon-Mars exploration program, which could culminate in human missions to Mars (President's Commission, 2004; NASA, 2004).

The rolling wave of Mars exploration has several implications for preventing the forward contamination of Mars. First, the number of planned Mars missions alone increases the potential for contamination. Second, the scope of activities conducted during surface missions and the number of potentially habitable environments to be explored is expected to increase dramatically, starting with the Phoenix mission to be launched in 2007. Missions such as the Mars Science Laboratory, ExoMars, Mars Sample Return, and the Mars Astrobiology Field Laboratory will all be specifically targeted to regions where scientists expect to confirm the past or present existence of water

[6]ExoMars is the flagship mission in the Aurora program.

(liquid or ice). The mission goals of ExoMars, Mars Sample Return, and the Mars Astrobiology Field Laboratory are also likely to include the search for extant or fossil martian life, and the makeup of instrument payloads on many future Mars missions is likely to focus on life detection. The sophistication and sensitivity of Mars lander instruments are evolving in response both to new technologies and information from previous missions, and instrument measurement sensitivities can be expected to improve with each subsequent mission.

Since publication of the 1992 NRC report on the forward contamination of Mars (NRC, 1992), 12 missions (orbiters and landers) have been sent to Mars, 6 of which have successfully completed their scientific objectives (see Chapter 1, Table 1.1). Knowledge of the planet continues to grow rapidly, and the need to revisit bioburden requirements on an ongoing basis is thus critical.[7] Chapter 4 describes the current scientific understanding of the Mars environment, particularly research on the potential for transient and long-lived liquid water—a key factor in considering the prospects for forward contamination.

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[7]The bioburden is the level of microbial contamination (total number of microbes or microbial density) in or on an item of interest, in this case, a spacecraft. Additional planetary protection terminology is defined in Box 1.1.

4

Environments on Mars Relative to Life

Currently, we do not know whether there is, or has ever been, life on Mars, nor do we know if there are environments on Mars today that can sustain life—terrestrial or martian. However, as knowledge of Mars grows and understanding of the planet matures, it is increasingly evident that there may currently be diverse environments on Mars that may be or might have been hospitable to life, either now or in the recent past. This chapter presents a snapshot of current understanding of the possibilities for potentially habitable martian environments. Analyses of new spacecraft data are revolutionizing many aspects of scientific understanding of Mars, including the possibilities for habitable environments. These possibilities could expand further or contract considerably as additional new data from Mars missions are acquired and analyzed. However, the general trend is now toward increasing prospects for habitability, a trend that should be factored into future policies regarding forward contamination.

Based on terrestrial experience, life has three minimum prerequisites: biogenic elements (such as carbon, nitrogen, and other elements critical for biological molecules), a utilizable source of energy, and liquid water (to provide the medium for biochemistry). The presence of life as we know it implies the presence of organic molecules, and many theories of the origin of life require certain prebiotic organics to be present in the environment prior to life's origin.[1]

An ideal habitable environment would have all three prerequisites continuously available. However, on Earth, life is able to flourish in environments where all three prerequisites are not continuously coincident in space and time (see Chapter 5). Therefore, consideration of environments on Mars that are relevant to life necessarily requires factoring in temporal variability, which may occur on daily, seasonal, interannual, and climatic (10^4 to 10^7 year) timescales. The next three sections outline current understanding of the three minimum prerequisites for martian habitability. The rest of the chapter discusses the implications for the definition of special regions[2] and the need for additional observations and studies.

[1]Organic molecules are molecules based on carbon bonded to hydrogen or nitrogen and perhaps other atoms: organic molecules can be produced by both biological and nonbiological processes. For a review of current thinking in orgins-of-life and astrobiology research, see Chyba and Hand (2005).

[2]For the COSPAR definition of special region, see Box 1.1.

BIOGENIC MATERIALS

Mars is known to be well endowed with most of the elemental building blocks of life, although the martian inventory of nitrogen, in particular, remains poorly understood (Mancinelli, 1996). Mars's inventory of these elements is being continuously supplemented through the influx of cometary and meteoritic material.[3] A significant fraction (1 to 10 percent) by weight of infalling material is in the form of organic material synthesized by abiotic processes. The annual delivery of reduced organic compounds to the martian surface, most of it due to interplanetary dust particles and micrometeorites, is estimated to be 2.4×10^8 g/yr (Flynn and McKay, 1990; Benner et al., 2000).

Present data on martian organics come from two sources. The first are the Viking pyrolysis gas chromatograph-mass spectrometer (GCMS) analyses of martian soils, which detected the solvents used to clean the instrument on Earth but found no traces of martian organics to the limits of the instrument's sensitivity (Biemann et al., 1977). The second source of data is that fraction of the organics contained in martian meteorites that is not due to terrestrial contamination (Jull et al., 1998; Becker et al., 1999). The organics identified in martian meteorites include polycyclic aromatic hydrocarbons (PAHs) and kerogens, some of which exhibit $^{13}C/^{12}C$ ratios similar to those observed in organics in primitive meteorites, suggesting that Mars may contain a record of extraterrestrial organic carbon compounds delivered by meteorites (e.g., Jull et al., 2000). The failure of the Viking GCMS to detect martian organics has been explained by (1) the absence of organics in the strongly oxidizing near-surface soil environment (Biemann et al., 1977), (2) insufficient instrument sensitivity (Glavin et al., 2001), (3) inability to achieve the high oven temperatures required to volatilize complex kerogen-like components (Becker, 2002), and (4) inability to detect the presence of the nonvolatile products of oxidative degradation such as the salts of organic acids (Benner et al., 2000).

Oxidants (such as hydrogen peroxide, H_2O_2) derive from photochemical processes in the martian atmosphere and from the interaction of solar ultraviolet radiation with surface minerals to form superoxides (Yen et al., 2000). However, Mancinelli (1989) has shown that certain Earth soil bacteria can survive much higher concentrations of H_2O_2 than are implied by the Viking measurements. Moreover, geologic processes have managed to preserve organics in terrestrial sedimentary rocks for billions of years (e.g., Foriel et al., 2004), despite oxidizing surface conditions. Therefore, it is unlikely that the presence of oxidants in the martian atmospheric and near-surface environments in itself represents an insurmountable challenge for martian habitability.

UTILIZABLE ENERGY

Earth's biota derive useful energy either from light or from chemical reactions. Sunlight is abundant at the surface of Mars, but in the absence of evidence for the absorption of sunlight by martian biota, most considerations of biologically useful energy on Mars have focused on chemical sources. Utilizable chemical energy becomes available when disequilibrium redox conditions are created in the environment by processes such as volcanism, chemical weathering, and atmospheric photochemistry. There is evidence that all three processes either currently occur, or have occurred recently, on Mars. Crater counts in the calderas of martian volcanoes suggest that that eruptions have occurred as recently as 2 million years ago (Neukum et al., 2004). The weathering of iron minerals has been considered as a potential energy source for an early martian biosphere (Jakosky and Shock, 1998), and like the crust of the Earth, martian meteorites show evidence of incomplete chemical weathering (Treiman et al., 1993). Photochemical processes in the martian atmosphere result in the ongoing production of reactive species, such as H_2, O_2, and CO (Nair et al., 1994) that could be an energy source for martian biota (Weiss et al., 2000; Summers et al., 2002). The relatively good agreement between the observed concentrations of these species and chemical models that do not include surface sinks (biotic or abiotic) has been used to place upper limits on the overall metabolic activity of hypothetical biota. Also, it is concluded that Mars's present-day biotic carbon flux

[3]Sufficiently large and fast impactors may eject much of their material to space and erode the martian atmosphere; see Melosh and Vickery (1989) and Chyba (1990).

can be at most 4×10^{-5} times that of Earth's (Weiss et al., 2000). These results suggest that distribution of hypothetical martian biota may not be limited by the availability of energy, but rather by the availability of liquid water.

The metabolic activity of a potential martian biosphere is one of several possible explanations for recent claims of the detection of roughly 10 ppb of methane in the martian atmosphere (Mumma et al., 2004; Krasnapolsky et al., 2004; Formisano et al., 2004). If this detection is correct, the approximately 300-year photochemical lifetime of methane in the martian atmosphere implies the presence of a significant and recent methane source. Candidate abiogenic methane sources include recent volcanism, hydrothermal sources, cometary and meteorite impacts, and the serpentization of basalt (Wallendahl and Treiman, 1999; Krasnopolsky et al., 2004; Formisano et al., 2004). Candidate biogenic sources include fossil biogenic methane diffusing out of the martian crust and ongoing methane production from a hypothetical martian biosphere (Farmer, 1996; Fisk and Giovannoni, 1999; Max and Clifford, 2000; Krasnopolsky et al., 2004; Formisano et al., 2004). The global atmospheric source strength of methane on Mars is less than one-millionth that of methane on Earth. This suggests that, if the amount of methane that appears to be present in the atmosphere is in equilibrium with its production rate (i.e., there is no net accumulation of methane in the subsurface), then the metabolic rate of any hypothetical martian biosophere is quite small. Neither the validity of this assumption nor the accuracy of the identification of atmospheric methane can currently be confirmed. If subsequent investigations were to verify the presence of methane and also confirm that it is of biological origin, the motivation and requirements for preventing the forward contamination of Mars would have to be thoroughly reassessed.

LIQUID WATER

On Mars today, the limiting requirement for habitability appears to be the presence of liquid water. The potential for forward contamination of Mars by Earth microorganisms is therefore closely tied to the existence, state, and distribution of water reservoirs across the planet. Thus, water is the key indicator for special regions (see Box 1.1) on Mars. Knowledge of the distribution and behavior of water in all its forms on Mars is at present incomplete, particularly with respect to liquid water.

The subsections below summarize current understanding of the total martian reservoir and discuss the prospects for liquid water in the deep and near subsurface.

The Total Martian Water Reservoir

Mars exhibits widespread evidence of extensive modification by the effects of impacts, volcanism, liquid water, ice and wind—processes that appear to have been more active in the planet's past. Evidence for a water-rich Mars is provided by the geomorphic interpretation of a long list of landforms (e.g., Carr and Schaber, 1977; Rossbacher and Judson, 1981; Carr, 1986, 1996; Squyres et al., 1992; Malin and Edgett, 2000, 2003) and by geochemical and sedimentary evidence, recently acquired by the Mars Exploration Rover (MER), of episodic inundation by shallow surface water at Meridiani Planum (Squyres et al., 2004a,b).[4]

The most persuasive geomorphic evidence for large amounts of water on Mars are the outflow channels—broad scoured depressions hundreds of kilometers long that exhibit braided and streamlined forms within their beds. The channels generally emerge abruptly from large areas of collapsed and disrupted terrain, the apparent result of a massive release of groundwater. The distribution, size, and range of ages of these features suggest that a significant body of groundwater was present on Mars throughout much of its geologic history and may still persist today (Baker, 1982; Tanaka, 1986; Tanaka and Scott, 1987; Carr, 1986, 1996; Baker et al., 1991).

Using a conservative estimate of the volume of water required to erode the outflow channels, and the likely extent of their subsurface source regions, Carr (1986) estimates that Mars may possess a planetary inventory of

[4]Meridiani Planum is a region of ancient (~4 Gya, i.e., 4 billion years ago) terrain located just a few degrees south of the martian equator.

water equivalent to a global ocean 0.5 to 1 km deep if uniformly distributed over the planet's surface. Because the peak in outflow channel activity appears to have occurred ~2 to 3 Gya (billion years ago) (Tanaka, 1986), it significantly post-dates the period when the most efficient mechanisms of planetary water loss (impact erosion and hydrodynamic escape) are thought to have been active (>4 Gya). Thus, it is expected that the vast bulk of this water still resides on Mars. Studies of recent (less than a few hundred million years old) martian meteorites further support this conclusion; there is evidence that the parent magma from which several of these meteorites were derived outgassed significant amounts of water (Dann et al., 2001).

Little of the 0.5 to 1 km estimated inventory of existing water is in the martian atmosphere or apparent at the martian surface. Only about 0.000001 percent of the inventory is found in the atmosphere (Farmer and Doms, 1979), while ~5 to 10 percent is thought to be stored as ice in the perennial polar ice caps and layered deposits (Clifford et al., 2000). That leaves ~90 to 95 percent of the total H_2O unaccounted for, almost all of which is thought to be stored as ground ice and groundwater within the planet's crust (Carr, 1996). To understand the distribution and state of water on Mars, one must understand the distribution and state of its subsurface water.

Deep Subsurface Water

A comprehensive geophysical survey for deep subsurface water on Mars has yet to be conducted. In its absence, knowledge of the distribution and state of deep subsurface water depends strongly on theoretical models and analogies to Earth. This distribution is likely to be influenced by an equivalent level of geologic complexity and the spatial variability of such crustal characteristics as lithology, structure, stratigraphy, porosity, permeability, ice content, mechanical strength, and thermal properties.[5]

The distribution of the two principal reservoirs of deep subsurface water on Mars—ground ice and groundwater—is thought to be determined by the thermal structure of the crust. Current mean annual surface temperatures on Mars range from ~154 kelvin (K) at the poles to ~218 K at the equator, with the geothermal heating, produced by the decay of natural radioactive materials in the crust, expected to result in increasingly warmer temperatures at depth. Consideration of the current best estimates of the planet's mean geothermal heat flux, about 15 to 45 milliwatts per square meter (mW/m^2) and the plausible range of freezing temperature (~252 to 273 K) of any saline groundwater that may be present at depth, suggests that the thickness of frozen ground (cryosphere) on Mars should vary from ~2.5 to 5 km at the equator to ~6.5 to 13 km at the poles (Fanale, 1976; Rossbacher and Judson, 1981; Kuzmin, 1983; Clifford, 1993). (See Figure 4.1.) However, natural variations in crustal heat flow, thermal conductivity, and the presence of potent freezing-point-depressing salts are likely to result in significant local differences in the thickness of frozen ground, when compared with estimates based on the assumption of latitudinally averaged values (Clifford and Parker, 2001; Travis et al., 2003).

At equatorial and midlatitudes on Mars, the low relative humidity of the atmosphere will tend to cause near-surface ground ice to sublime at a rate dependent on the local surface temperature and diffusive properties of the crust (Smoluchowski, 1968; Clifford and Hillel, 1983; Fanale et al., 1986; Mellon and Jakosky, 1993). Depending on the nature of these properties, their variation with depth, and the potential for replenishment from any deeper reservoir of subpermafrost groundwater, the result may be the progressive loss of ice by sublimation from the local regolith to depths that range from centimeters to as much as a kilometer (Smoluchowski, 1968; Clifford, 1993, 1998; Mellon and Jakosky, 1995).

If the martian inventory of water exceeds what can be stored as ice within the pore volume of the cryosphere, then the bulk of the excess will be present as a liquid, saturating the lowermost porous regions of the crust—which, as illustrated in Figure 4.1, may reside at depths that range from about 3 to as much as 13 km below the martian surface, depending on the local surface temperature and heat flow (Clifford, 1993; Carr, 1996). Given a large-scale crustal permeability comparable to that of Earth, and the lack of any recent rainfall, the influence of gravity should

[5]The expected distribution and state of subsurface water on Mars, as well as the large-scale physical, thermal, and hydraulic properties of its crust, have been discussed by Rossbacher and Judson (1981), Kuzmin (1983), Squyres et al. (1992), Clifford (1993), Carr (1996), and Clifford and Parker (2001), among others.

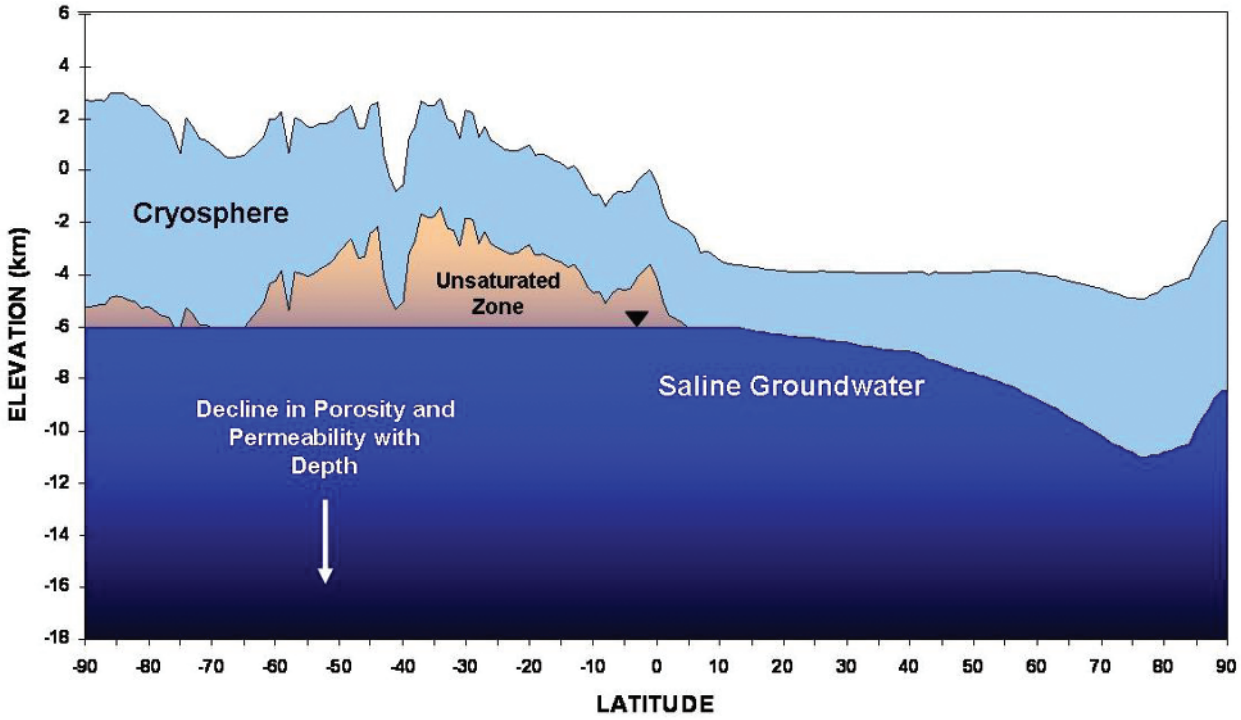

FIGURE 4.1 Hypothetical pole-to-pole cross section of the present day martian crust along 157° longitude, illustrating the potential relationship between surface topography, ground ice, and groundwater. The depicted thickness of frozen ground (the cryosphere) assumes the present latitudinal range of mean annual surface temperatures (~154 to 218 K), a column-average thermal conductivity of 2 W m^{-1} K^{-1}, and a global mean geothermal heat flux of 30 mW m^{-2} (modified from Clifford and Parker, 2001).

result in a present-day groundwater system that is in effective equilibrium (i.e., characterized by a relatively flat water table)—except where it may be locally perturbed by tectonic, seismic, or thermal processes. Because of the low porosity expected at depth,[6] comparatively little water is required to produce a groundwater system of substantial extent. Therefore, if a subpermafrost groundwater system is present on Mars, it may underlie much of the planet's surface—although the extent to which it may be interconnected is unknown (Clifford, 1993; Carr, 1996).

The distribution of ground ice is expected to follow the thermal structure of the crust, whereas the distribution of groundwater, under the influence of gravity, will drain and saturate the lowermost porous regions present at depth. For this reason, the vertical distance separating these subsurface reservoirs may vary considerably, such that the intervening unsaturated zone is maximized in regions of high elevation and minimized, or absent, at lower elevations (see Figure 4.1). Within the unsaturated zone, water vapor will tend to diffuse from the higher-temperature (higher-vapor-pressure) depths to the colder (lower-vapor-pressure) region just below the base of the cryosphere. As this moisture-laden air rises and cools, some of the vapor will condense, creating a low-temperature hydrothermal convection system of rising vapor and descending liquid condensate. Such a system may have resulted in complicated variations in saturation state between the base of the cryosphere and the regional ground-

[6]Porosity decreases exponentially with depth because the pressure of the overlying rock collapses the pores that might otherwise be present.

water table and may also have contributed to the development of the underlying groundwater into a highly mineralized brine (Clifford, 1993; Carr, 1996).

Although the outflow channels provide persuasive evidence that Mars once possessed a sizable inventory of subpermafrost groundwater, it is possible that today such a reservoir no longer survives—a potential consequence of the cold-trapping of a once-large inventory into the pore volume of the thickening cryosphere, as the planet's internal heat flow declined with time (Clifford and Parker, 2001). Thus, the present state of deep subsurface water on Mars is bracketed by two extremes: one in which a small planetary inventory, combined with the progressive cooling of the crust, has eliminated any persistent reservoir of groundwater, and another in which the planetary inventory is sufficiently large that a sizable reservoir of liquid may still survive at depth over much of the planet.

Near-Surface Water

Near-surface water on Mars is defined here as any water—whether present as ice, liquid (including brine), absorbed on mineral surfaces, or as vapor—that exists within the several hundred meters of the planet's surface, whether above, below, or on. Scientists further define two specific zones within the shallow subsurface where water may potentially be exchanged with the atmosphere in response to variations in atmospheric water vapor concentration and surface temperature: (1) the diurnally active layer, which extends down to a depth of ~20 cm, and (2) the seasonally active layer, which includes the top ~2 to 3 m of the regolith. These zones represent the approximate depths at which the amplitudes of the diurnal and seasonal temperature waves decay to zero. It is difficult for atmospheric water vapor to diffuse below the depth of the seasonally active layer because it would be acting against the geothermal temperature gradient, which causes water to diffuse from the warmer (higher-vapor-pressure) depths to the colder (lower-vapor-pressure), shallower regions of the crust.

As defined above, near-surface water may be found in the atmosphere, on the surface, and below the surface. The distribution and state of water in the near-surface region of Mars today are determined by a range of properties and processes that to a large extent are analogous to those that operate in cold dry regions on Earth. Cold soils that are in good diffusive contact with the atmosphere[7] act as local cold traps for water vapor and can accumulate surface and subsurface ice. As this ice warms, it acts as a local source of water vapor, and it can also melt to form transient liquid water if local atmospheric and temperature conditions permit. The overall behavior of the martian global water cycle is determined by the combined, time-varying influences of local sources and sinks, and the circulation and transport of water by the martian atmosphere (Jakosky et al., 1997; Richardson and Wilson, 2002).

As on Earth, the distribution and behavior of near-surface water on Mars are likely to exhibit substantial spatial and temporal variability. This is especially true in the subsurface, where local differences in the thermal, diffusive, and physical properties of the soil can exert a substantial influence on the exchange and retention of H_2O (Clifford and Hillel, 1983; Fanale et al., 1986; Mellon and Jakosky, 1993; Jakosky et al., 1995). Indeed, given geologically reasonable variations in the actual values and spatial distribution of soil properties, it is possible for near-surface liquid water and ice to become diffusively isolated from the atmosphere, even at depths as shallow as several centimeters. Examples of low-permeability materials capable of producing this effect include sulfate-cemented regolith, a layer of very fine clay, or rock. In such instances, liquid water and ice could survive in disequilibrium with the water vapor content of the atmosphere for billions of years (e.g., see Smoluchoski, 1968). Unfortunately, for the case of subsurface water on Mars, researchers are lacking many of the key observables necessary to understand and quantify fully its distribution and behavior.

The next four subsections outline current understanding of some of the key known near-surface water reservoirs. Possibilities for liquid water in the near-surface environment are then discussed.

[7]"Diffusive contact with the atmosphere" means that the gaseous permeability of the regolith is sufficient to allow water vapor to migrate into and out of the regolith, in essential equilibrium with its concentration in the atmosphere, in response to diurnal and seasonal temperature changes.

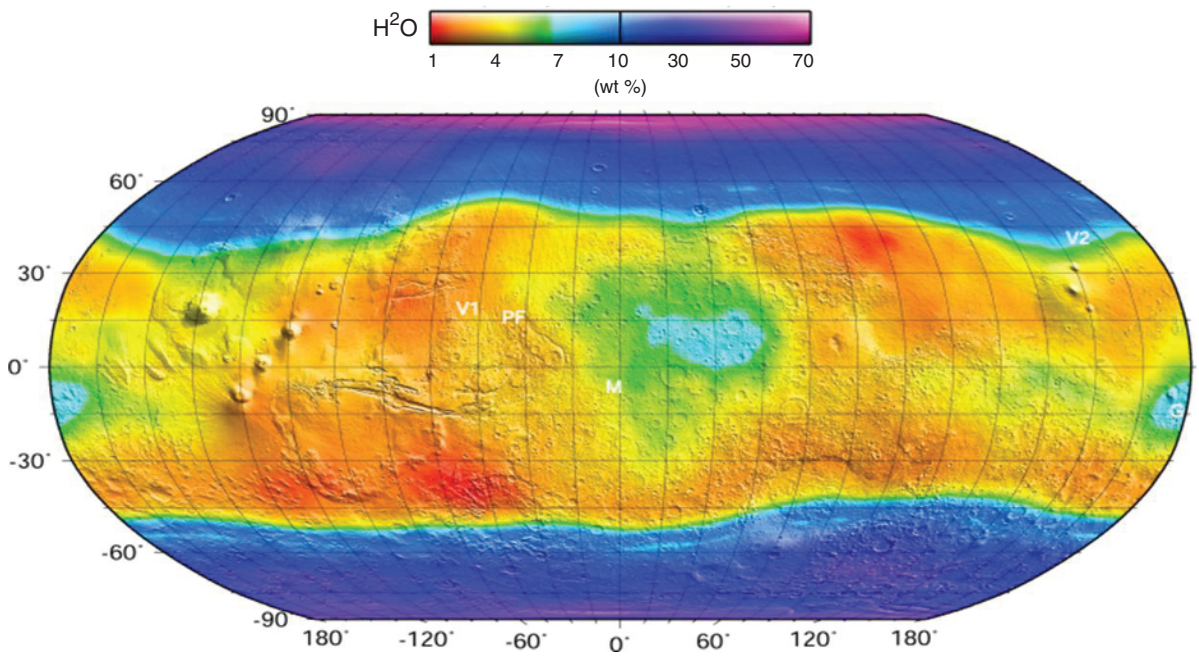

FIGURE 4.2 Global distribution and abundance of near-surface (top meter) hydrogen on Mars as inferred from the Mars Odyssey gamma ray spectrometer (GRS) data. Also shown on the map are the Viking 1 and 2 (V1 and V2), Pathfinder (PF), and Mars Exploration Rover Meridiani (M) and Gusev (G) landing sites. SOURCE: Courtesy of the GRS instrument team, Lunar and Planetary Institute, University of Arizona.

Near-Surface Ground Ice

Near-surface ground ice represents the most significant and most widely distributed near-surface martian water reservoir. Because thermal conduction from the planet's interior results in generally increasing temperatures with depth, the near-surface regions of Mars are actually the coldest places on the planet; therefore, they should be effective long-term cold traps for martian water (Clifford, 1991). The expected distribution of near-surface ground ice has been predicted by various models over the past 40 years (e.g., Leighton and Murray, 1966; Fanale, 1976; Fanale et al., 1986; Mellon and Jakosky, 1993). Those models show generally good agreement with global near-surface hydrogen abundance measurements made by the Mars Odyssey gamma ray spectrometer (GRS) instrument (see Figure 4.2) (Boynton et al., 2002).

The GRS high-latitude data are usually interpreted in the context of a simple two-layer model, where an ice-rich (>60 percent by volume) layer is assumed to underlie a desiccated layer of variable thickness. If taken literally, this model implies that the depth to ground ice varies from ~13 cm near the poles to ~50 cm at 40 to 50° latitude (Boynton et al., 2002). However, with a surface resolution of ~1 × 10^5 km^2, the hydrogen abundances determined from the GRS data are averaged over areas that are many orders of magnitude larger than the scale of variability observed in the physical and thermal properties of the planet's surface (Malin and Edgett, 2001; Christensen et al., 2003)—properties that may have a significant influence on the local distribution of near-surface ground ice. Thus, what appears as a uniform distribution of ice at a resolution of 10^5 km^2 may exhibit considerable variability at a scale of 1 km^2, such that regions possessing very high concentrations of near-surface ice may be interspersed with smaller regions that are ice-poor.

In the equatorial region below 40° latitude, the GRS data indicate that Mars is not completely dry. In this latitude range, GRS measures bulk soil water contents ranging from 2 to 8 percent. Mean equatorial annual surface

temperatures are high enough, and the concentration of atmospheric water vapor low enough, that near-surface ground ice is unstable if it is in diffusive equilibrium with the atmosphere. However, here too, small-scale variations in physical and thermal properties may contribute to substantial differences in the extent and magnitude of local desiccation. Thus, the lower abundance of hydrogen observed at mid- to equatorial latitudes by the GRS instrument can be interpreted in several ways: (1) it may reflect a region that is uniformly devoid of near-surface ice (at least, within the top meter) but that exhibits adsorbed water and hydrated minerals, as observed at the Mars Exploration Rover (MER) landing sites (Squyres et al., 2004a,b), by the Mars Express Orbiter OMEGA instrument (Bibring et al., 2005), and by the Viking Lander 1 GCMS instrument (Biemann et al., 1977)—the abundance of which could vary over large scales; (2) it may consist of broad areas where the top meter of the regolith is ice-free, but where the diffusion-limiting properties of the local soil have permitted shallow ice to survive over smaller regions (and, perhaps, more widely at depths >1 meter); or (3) it may be attributable to a combination of both explanations.

The Martian North and South Residual Polar Caps

The martian north and south residual polar caps also represent significant near-surface water reservoirs. Together, they have an exposed surface area roughly the size of the Greenland Ice cap and have been estimated to be ~3 to 5 km thick (Smith et al., 1999; Johnson et al., 2000). They contain exposed scarps that display continuous fine-scale layering that may be evidence for astronomically forced climate variability (see Figure 4.3) (Laskar and Robutel, 2004), as well as a variety of other geomorphic features suggesting recent glacial and/or aeolian activity (Thomas et al., 2000; Fishbaugh and Head, 2001).

Seasonal Surface Water Deposits

Seasonal surface water deposits occur when water ice is condensed directly on the surface in the form of frost, or deposited onto the surface in the form of snow. There is evidence for the existence of seasonal water deposits (Kahn, 1990), particularly at high latitudes where water is cold-trapped onto the seasonal solid carbon-dioxide deposits that constitute the martian seasonal polar caps (Bibring et al., 2005). The depths of seasonal water ice deposits on Mars under present climatic conditions are estimated to be on the order of tens of microns (Mischna et al., 2003). Figure 4.4 depicts a winter panorama at the Viking 2 landing site showing the accumulation of surface condensation.

Atmospheric Water

Atmospheric water is a minor constituent of the atmospheres of Mars and Earth, but it plays important roles in the climate systems of both planets. The water-vapor-holding capacity of the martian atmosphere is limited by the saturation vapor pressure, which at low martian temperatures is less than 1,000 times lower than typical values on Earth. Water on Mars achieves mobility in the near-surface region through atmospheric transport in the vapor phase and by the precipitation of condensed phases. Figure 4.5 shows measurements of seasonal variations in atmospheric water vapor and water ice clouds from the Mars Global Surveyor (MGS) orbiter thermal emission spectrometer (TES) instrument (Smith et al., 2001). Atmospheric water shows strong seasonal variations associated with the holding capacity of the martian atmosphere and the condensation and sublimation of water from near-surface reservoirs such as ground ice, adsorbed water, and surface ice.

Liquid Water Stability

The properties and processes that determine the stability of liquid water in the martian near-surface environment are analogous to those on Earth. Figure 4.6 shows a phase diagram for pure water. At total pressures above the triple point of 6.1 mbar, pure liquid water can exist on the surface of Mars at temperatures above the freezing temperature of ~273 K and below the boiling temperature of ~283 K, where its saturated vapor pressure exceeds

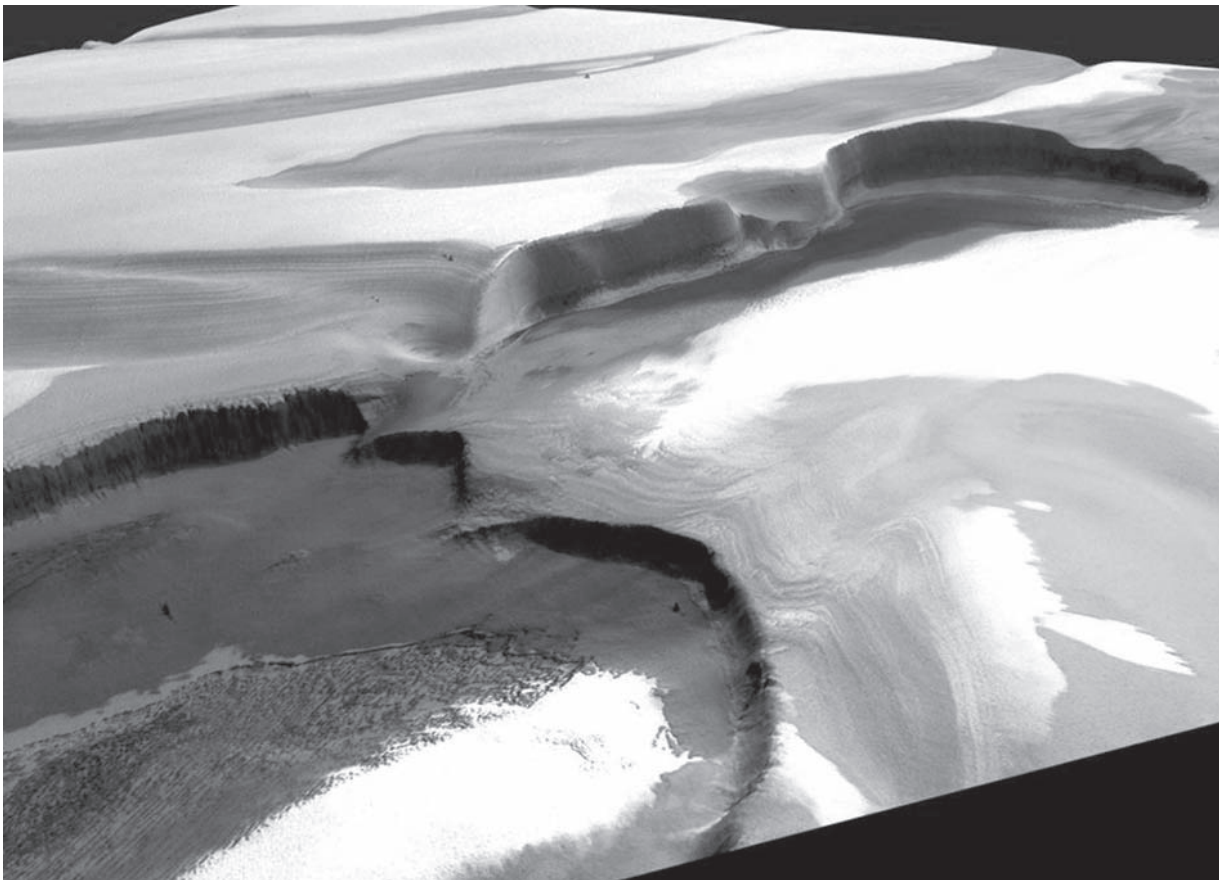

FIGURE 4.3 Mars Express high-resolution stereo camera three-dimensional image of the martian north polar residual cap showing surface ice, layered deposits, scarps, and sand dunes. SOURCE: Courtesy of European Space Agency/Deutsches Zentrum für Luft- und Raumfahrt e.V./Freie Universität Berlin-G. Neukum.

the total atmospheric pressure. Because of the low partial pressure of water vapor in the martian atmosphere, liquid water in diffusive contact with the atmosphere will evaporate rapidly, and so the stability of liquid water at the surface today is at best transient. The relatively narrow temperature range at which pure water is transiently stable at the martian surface is controlled by the atmospheric surface pressure, which exhibits both geographic and seasonal variations (Haberle et al., 2001). Figure 4.7 shows general circulation model (GCM)[8] calculations of current martian annual maximum surface pressures and annual maximum surface pressures. Figure 4.8 shows GCM calculations of the locations and length of time in a Mars year in which martian surface temperatures are estimated to be above the triple point and below the boiling point (Haberle et al., 2001).

The results shown in Figures 4.6, 4.7, and 4.8 assess the potential stability of liquid water to freezing and boiling, but they do not address the issue of evaporation and stability in detail. In general, water is stable to evaporation only when the local partial pressure of atmospheric water vapor equals the vapor pressure of the liquid. That situation rarely occurs on the surface of Earth for extended periods, so consistent with common

[8]GCM in this case refers to a numerical representation of the atmosphere and its phenomena over all of Mars.

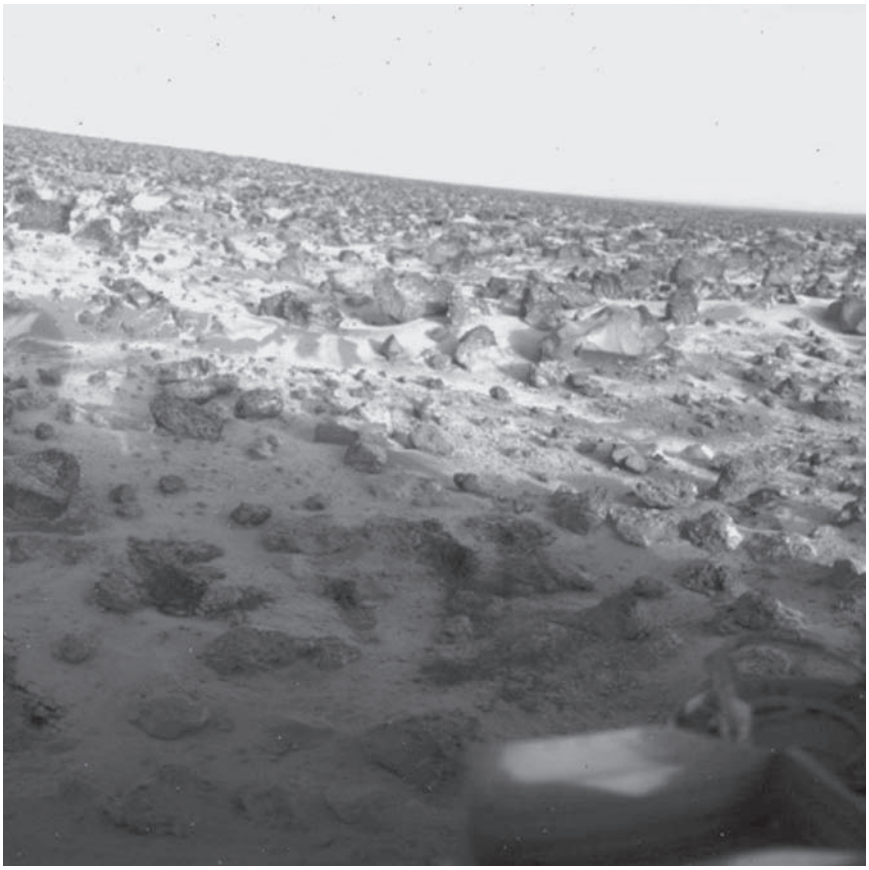

FIGURE 4.4 Winter scene at the Viking 2 landing site at Utopia Planitia, +47.96°N latitude. SOURCE: Courtesy of NASA.

experience, liquid water tends to evaporate unless it is actively replenished. Similarly, transient liquid water is also possible on the surface of Mars, although as is discussed, there is no direct evidence for its existence, much less its distribution.

While pure liquid water freezes at temperatures below 273 K, there are two mechanisms that could depress both the freezing temperature and the corresponding minimum pressure required for liquid water stability. The first is the well-documented presence of thin films of liquid water on soil and ice grain boundaries that can persist down to temperatures as low as ~20 K below the freezing point (Anderson and Tice, 1973; see also Figure 4.9). It has been suggested that such water films could extend the geographic range of survivability for martian biota (Jakosky et al., 2003).

A second mechanism for depressing the freezing point is the presence of dissolved solutes (Brass, 1980; Clark and Van Hart, 1981; Knauth and Burt, 2002). Observations at the MER landing sites (Squyres et al., 2004a,b) and by the Mars Express Orbiter OMEGA spectrometer (Bibring et al., 2005) suggest the widespread distribution of sulfates and other evaporite minerals on the martian surface. The sulfate content of rocks and soils at the MER Meridiani landing sites ranges from 5 to 25 percent by weight (Rieder et al., 2004). Models predict eutectic[9]

[9]A eutectic is a mixture of two or more compounds that has a lower melting point than do its constituents.

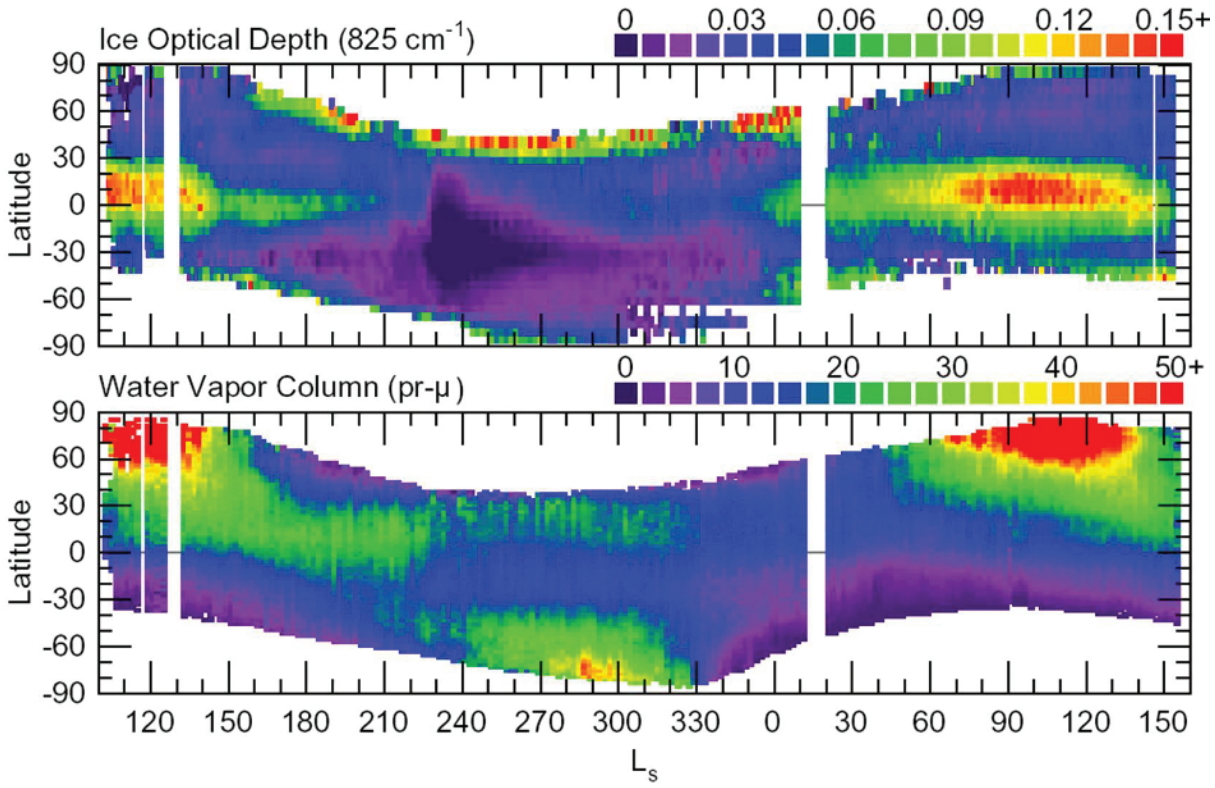

FIGURE 4.5 Mars Global Surveyor thermal emission spectrometer observations of atmospheric water ice cloud optical depth (top) and column water vapor (bottom) as a function of martian season (L_s). SOURCE: Smith et al. (2001). Copyright 2001 American Geophysical Union. Reproduced by permission of American Geophysical Union.

freezing point temperatures for plausible martian brines of 225 K or lower (see Figure 4.10) (Brass, 1980; Knauth and Burt, 2002; Madden and Bodnar, 2002). If brines with high salt concentrations are present in the near-surface environment, then there is the potential for near-surface liquid water almost everywhere on the planet.

In general, the available information paints a highly uncertain picture regarding the present existence of liquid water on Mars. From a thermodynamic standpoint, pure liquid water can exist only in restricted geographic locations during restricted time periods. However, such water would evaporate rapidly into the dry martian atmosphere, and it would have to be replenished in order to be present over many diurnal or annual cycles. Liquid water in the near-subsurface faces similar challenges, but the requirements for resupply could be significantly reduced by the presence of diffusive barriers such as fine-grained soil, duricrust, ice, or rocks. The recent verification of the presence of high concentrations of water-soluble minerals on Mars greatly increases the prospects for liquid water environments enabled by freezing-point depression. Researchers also know that, at least to the spatial resolution of the GRS instrument, water molecules are globally distributed throughout the martian near-surface environment, at spatially averaged concentrations that exceed 1 percent. Taken together, these facts do not add up to a convincing case for or against the presence of liquid water. Instead, they underscore the need for additional measurements and analysis. Very cold yet liquid water environments could potentially currently exist at many locations in the martian near-surface.

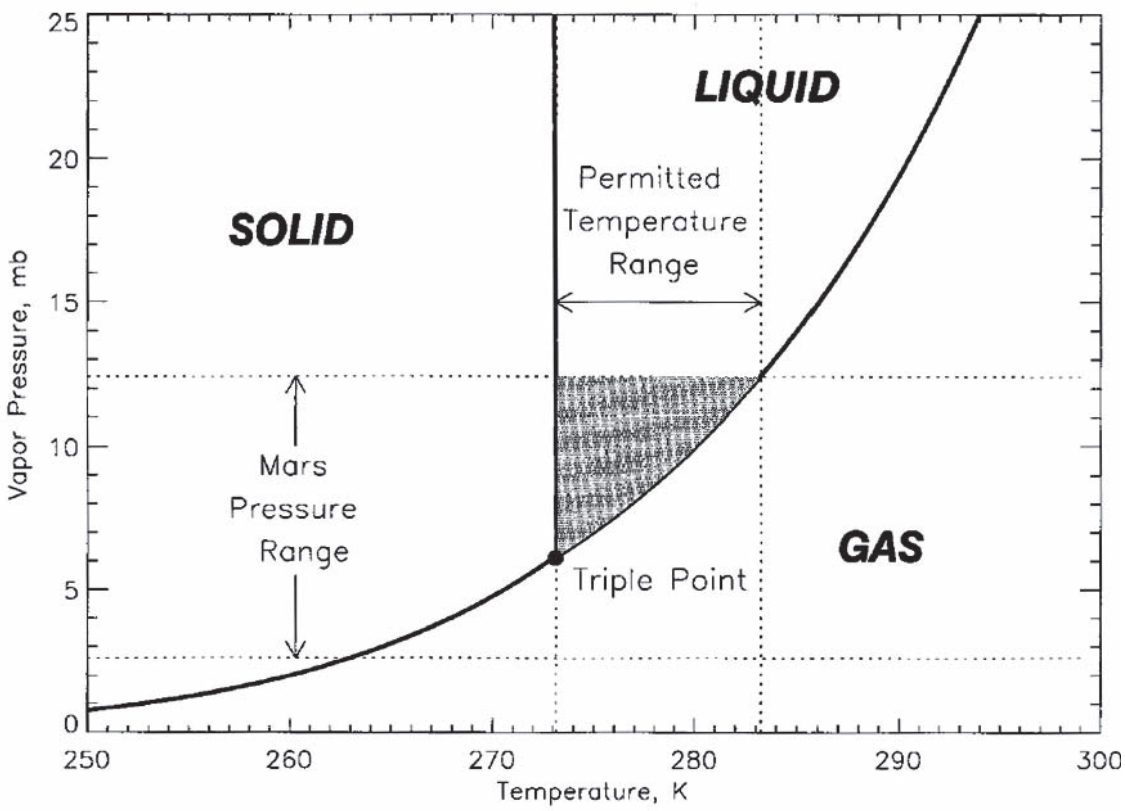

FIGURE 4.6 Phase diagram for pure water. The shaded region shows the conditions under which pure liquid water on the surface of Mars today is transiently stable. SOURCE: Haberle et al. (2001). Copyright 2001 American Geophysical Union. Reproduced by permission of American Geophysical Union.

Potential Influence of Obliquity-Induced Climate Change

The preceding discussion assumes global environmental conditions characteristic of the present martian climate. However, the presence of both large- and fine-scale layering in the martian polar stratigraphy suggests that the climate has undergone quasiperiodic change in response to a variety of astronomical variables operating on many different time scales. Of these, the martian obliquity exerts the greatest influence. The obliquity oscillates about its current mean value (i = ~25°) with a period of 1.2×10^5 years. The amplitude of this oscillation also varies and is modulated with a period of 1.3×10^6 years (Ward, 1992). At low obliquity, both seasonal temperature fluctuations and mean annual polar temperatures are at a minimum. This situation is reversed at times of high obliquity, when summers of continuous illumination alternate with dark winters to produce both extreme seasonal variations and higher mean annual temperatures at the poles.

Recent studies have demonstrated that the evolution of the martian obliquity is chaotic, varying from ~0° to 60° (Touma and Wisdom, 1993; Laskar and Robutel, 1993; Laskar et al., 2004). This behavior places an upper limit of several million years on how far back (or forward) the present obliquity can be reliably extrapolated. At obliquities above ~54° the mean annual insolation at the poles actually exceeds that at the equator (Ward, 1992). However, even at lower obliquities, maximum daytime temperatures at the poles can still exceed 273 K throughout much of the spring and summer (Toon et al., 1980; Pathare and Paige, 1998). Under these conditions, high-latitude

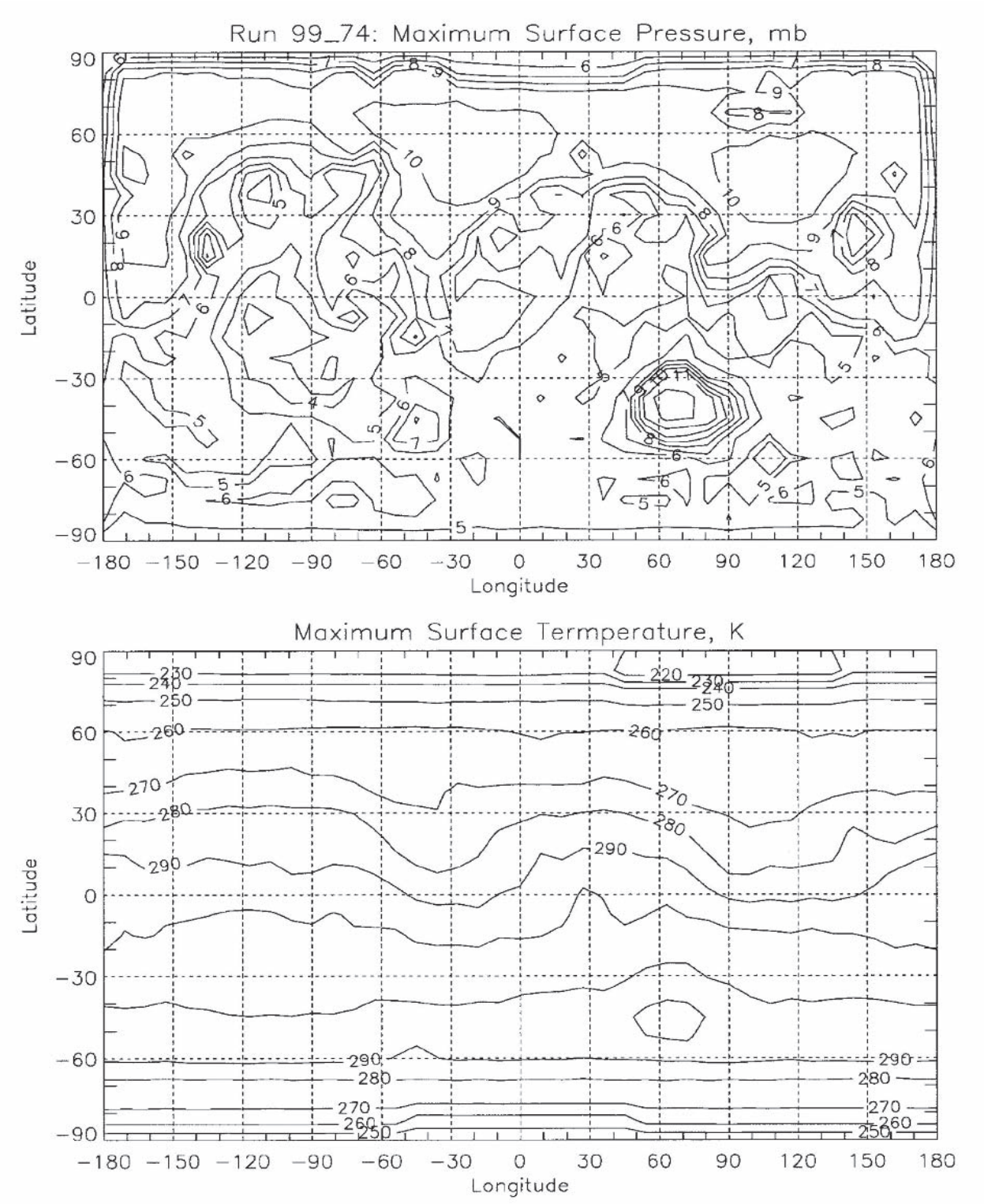

FIGURE 4.7 General circulation model calculations of current martian annual maximum surface pressures (top) and annual maximum surface temperatures (bottom). SOURCE: Haberle et al. (2001). Copyright 2001 American Geophysical Union. Reproduced by permission of American Geophysical Union.

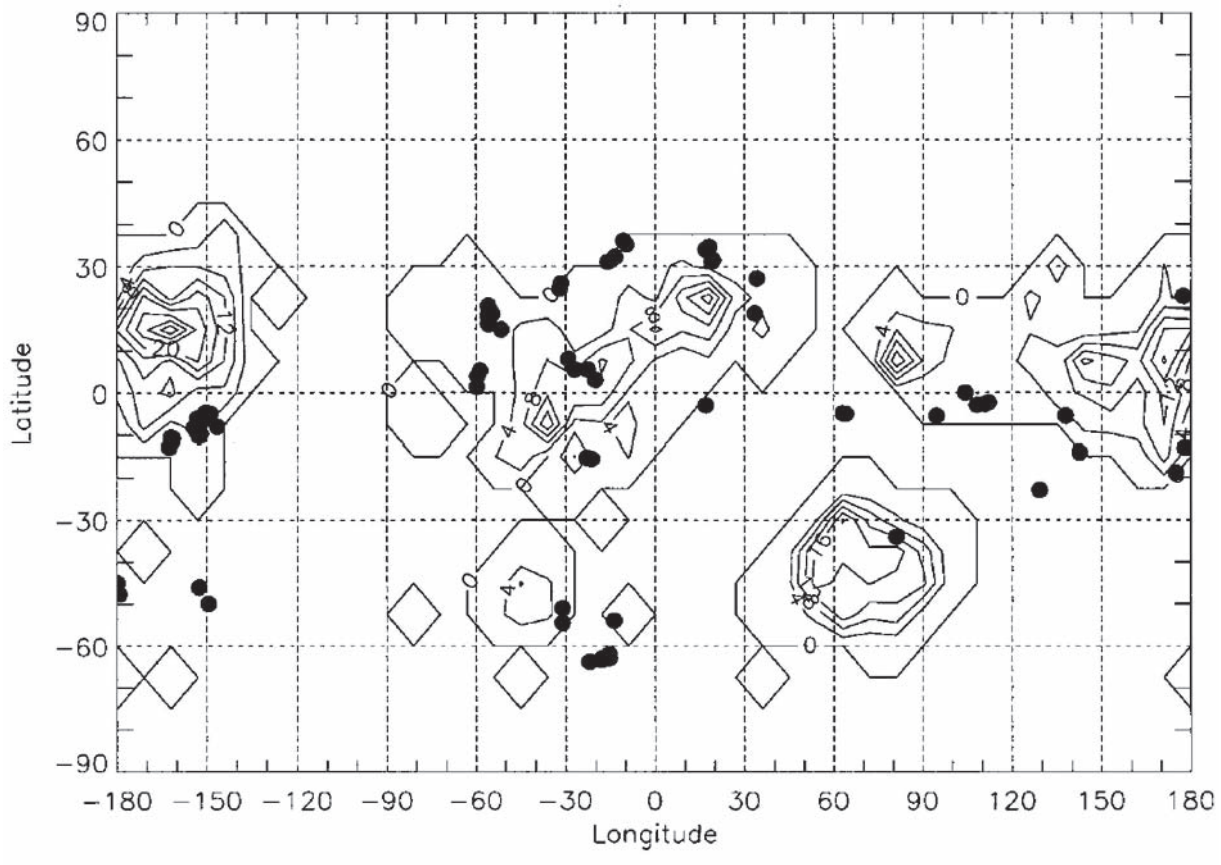

FIGURE 4.8 General circulation model calculations of the locations and length of time (in sols or martian days) during a Mars year at which martian surface temperatures are estimated to be above the triple point and below the boiling point (Haberle et al., 2001). Also shown (black dots) are the locations of possible Amazonian paleolakes from Cabrol and Grin (2001). SOURCE: Modified from Haberle et al. (2001). Copyright 2001 American Geophysical Union. Reproduced by permission of American Geophysical Union.

sublimation rates for exposed ice may reach ~0.1 m/yr averaged over a single obliquity cycle (Jakosky et al., 1995). There could also be widespread melting of the polar ice, as well as high-latitude snow packs and near-surface ground ice (Pathare and Paige, 1998; Costard et al., 2002; Christensen, 2003), creating episodic liquid water environments, lasting for days or many months, that would be repeated on an annual basis for as long as the high-obliquity phase of the cycle persisted (up to ~10^4 years). Theoretical studies suggest that such conditions might recur on 10^6- to 10^7-year timescales.

A CATALOG OF POTENTIALLY SPECIAL REGIONS

As mentioned above, COSPAR currently defines a special region on Mars as "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms. Given current understanding, this applies to regions where liquid water is present or may occur." (See Table 2.1.) The following is a catalog of potentially accessible special regions on Mars:

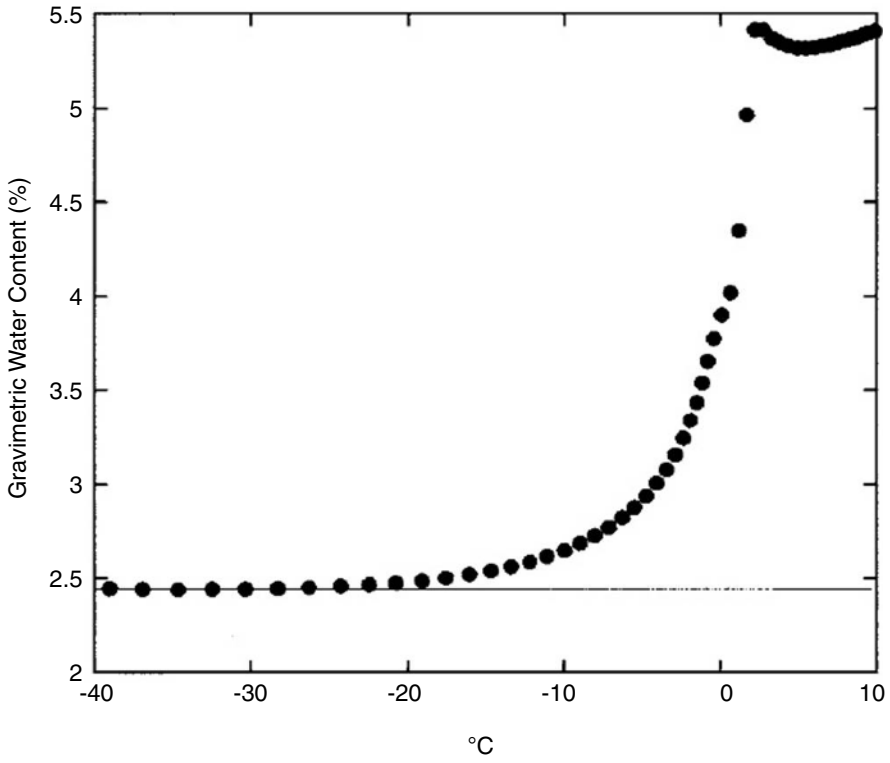

FIGURE 4.9 Liquid water content measurements in loam soil as a function of temperature. The minimum water content represents unfrozen adsorbed water (Jakosky et al., 2003). NOTE: "Loam soil" is a porous soil composed of sand, silt, clay, and organic matter.

1. *Near-surface liquid water.* During the summer seasons at noon, temperature and pressure conditions at the surface in low-altitude equatorial and southern latitudes may permit the transient occurrence of pure liquid water at the surface (see Figure 4.8). Over much of the rest of the planet, surface water films and liquid brines may potentially exist during the warmer parts of the day. Because the vapor pressure of condensed phase water increases with temperature, there will be a strong tendency for warm liquid water to evaporate and ultimately recondense as ice in colder areas. Therefore, the repeatable formation of ephemeral liquid water on Mars will generally require a source of water ice that can be replenished on diurnal, seasonal, and climatic timescales (Hecht, 2002). Environments that are isolated from diffusive contact with the atmosphere, such as the interiors of rocks or regolith overlain by duricrust, can form liquid water whenever melting temperatures are reached. Even with liquid water periodically present, on a mean annual basis, these will be cold environments.

2. *Geothermal hot spots.* Although regions of current active volcanism or enhanced heat flow have not yet been identified, if they exist such regions and their immediate surroundings may well be special. By analogy to Earth, martian hot spots could provide abundant utilizable energy and liquid water.

3. *Segregated ground ice.* Geologic evidence for extensive and repeated flooding by outflow channel activity (Baker, 1982; Carr, 1996) suggests that massive ice deposits, preserved at comparatively shallow depth beneath a protective cover of lava flows and aeolian sediment, may now reside beneath the northern plains (Carr, 1990; Clifford and Parker, 2001; Murray et al., 2005). Although the case for such deposits remains controversial, it is conceivable that the volatile stratigraphy of the northern plains is quite complex, having potentially been built up through multiple episodes of flooding, freezing, sublimation, and burial. The presence of embedded solutes or

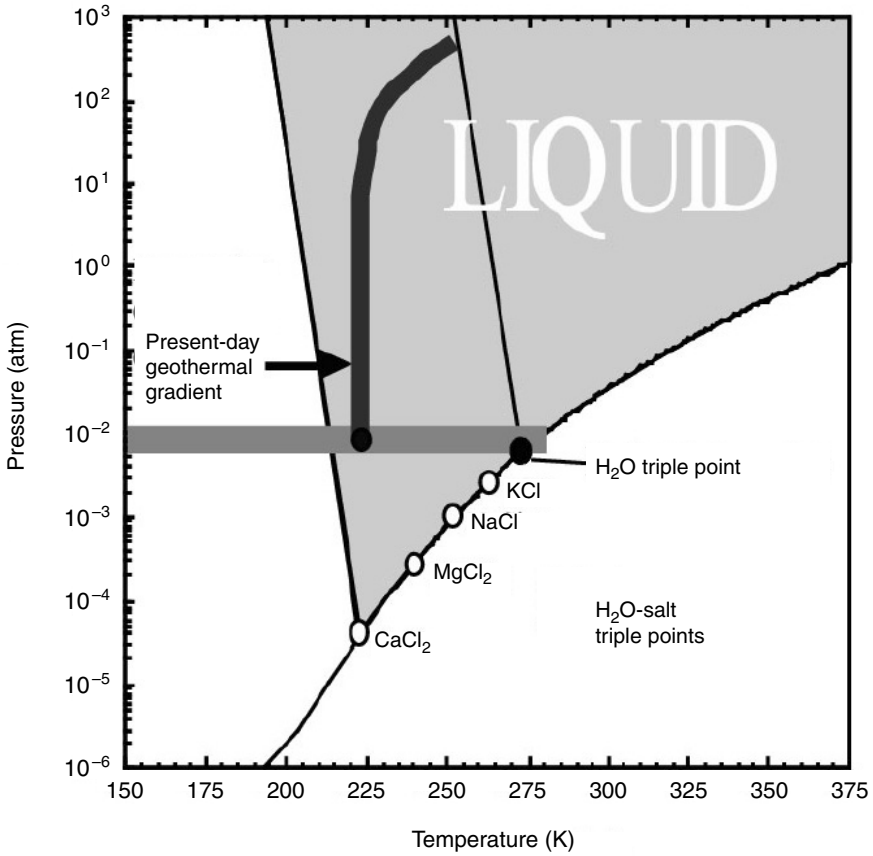

FIGURE 4.10 Calculated eutectic triple points for four molal water-salt compositions (Madden and Bodnar, 2002). SOURCE: Courtesy of R.J. Bodnar.

pockets of brine, the purposeful or accidental melting of massive ice as a consequence of exploration activities, or (on 10^5-year timescales) high obliquities could result in the production of substantially more liquid water where segregated ground ice occurs than would be produced for dispersed ice in soil under equivalent environmental conditions.

4. *Ice-rich frozen ground.* The GRS data shows that near-surface soil in high-latitude regions is nearly saturated with water ice. If some of this ice has melted in the recent past, then it likely contains high concentrations of solutes derived from adjacent martian rocks and soil, which could significantly depress melting points. Current observations cannot rule out the diurnal or seasonal melting of frozen brines in the near-surface environment.

5. *Polar caps and surface ice*. Dark regions on the north and south residual polar caps achieve seasonal maximum temperatures that could permit the presence of ephemeral water in the form of surface films and brines. Recent Mars Express OMEGA observations have revealed the presence of sulfate minerals in the dark polar sand sea adjacent to the north residual cap that indicate the past presence of at least ephemeral liquid water (Langevin et al., 2005). Theoretical models have shown that surface ice deposits containing small quantities of dust may support melting under certain circumstances (Clow, 1987). The melting of recent surface ice at midlatitudes is one of several explanations put forward to explain the origin of gullies (Christensen, 2003).

6. *Subpermafrost groundwater*. If the martian inventory of water exceeds what can be stored as ice within the pore volume of the cryosphere, then the bulk of the excess will be present as a liquid, saturating the lowermost

porous regions of the crust. The zonally averaged depth to the bottom of the cryosphere is estimated to be ~3 to 13 km, varying from its lowest to highest values with increasing latitude. These estimated depths are not well constrained by observations and are likely to exhibit significant variations due to localized differences in crustal thermal conductivity and heat flow. In areas of exceptionally high geothermal heat flow, or where potent freezing-point-depressing salts are abundant in the crust, near-surface environments may exist with possible hydrologic connections to a deeper and more widespread reservoir of subpermafrost groundwater.

7. *Gullies.* Mars exhibits widespread evidence for gully landforms on crater walls and other steep slopes that have been interpreted as geomorphic evidence for recent seepage and runoff of liquid water (Malin and Edgett, 2000). The timescales over which martian gully features have been hydrogically active is currently not well constrained, but it has been suggested that large-scale obliquity variations may have resulted in sufficient solar heating to cause localized melting of ground ice on million-year timescales (Costard et al., 2002). Although the mechanisms for gully formation on Mars are controversial (see Appendix F), gullies are likely to be regions where liquid water has a higher probability of occurring. Therefore, gullies and other landforms that suggest the presence of recent liquid water hold special significance for preventing forward contamination.

Taken together, the foregoing catalog of potentially special regions on Mars covers the entire surface of the planet. No specific region or range of latitudes, including the equatorial region, can currently be excluded, given available observations and current understanding. While certain regions on Mars may have a lower probability of being special than others, sufficient uncertainty remains, especially at smaller spatial scales, to warrant caution. A greater understanding of the distribution and state of subsurface (especially near-subsurface) water on Mars is needed. How this dilemma could be mitigated by upcoming exploration is discussed below.

TECHNIQUES FOR ASSESSING THE DISTRIBUTION AND STATE OF SUBSURFACE WATER ON MARS

Substantial uncertainties remain regarding the amount and distribution of subsurface water on Mars, yet these factors are of great importance for evaluating the potential for forward contamination of the martian environment by microbes carried on spacecraft. This section considers techniques currently available or soon to be available for providing an improved assessment of these factors.

Theoretical Modeling

The earliest efforts to model the stability and distribution of ground ice on Mars made many simplifying assumptions in order to focus on large-scale average effects.[10] More recent investigations (Paige, 1992; Mellon and Jakosky, 1993, 1995) have examined how local variations in the radiative and thermophysical properties of the surface could lead to significant geographic differences in ground ice stability. These investigations suggest that in some regions, under favorable conditions, fossil ground ice might survive at shallow depth even at the equator.

The acquisition of high-spatial-resolution thermal data alone is not sufficient to allow the prediction of the present local distribution of ground ice, because the thermal properties of the regolith cannot be extrapolated with any confidence beyond the depth that experiences diurnal temperature variations (essentially the top 10 to 25 cm). In fact, knowledge of many fundamental characteristics of the martian near-surface is poor, especially with respect to how the thermal and diffusive properties of the crust vary both geographically and at depth. Such variations play a determining role in the evolution and vertical distribution of ground ice.

[10]Leighton and Murray (1966), Fanale (1976), and Farmer and Doms (1979) considered the distribution of ice in equilibrium with the zonally averaged mean annual surface temperature and water vapor content of the atmosphere. Smoluchowski (1968), Clifford and Hillel (1983), and Fanale et al. (1986) examined the stability and evolution of ground ice under disequilibrium conditions, investigating how plausible values of regolith thermophysical and diffusive properties might influence the latitudinal stability of ground ice in an otherwise homogeneous crust.

Drill cores on Earth, lunar soil profiles, and high-resolution images of the walls of the canyon Valles Marineris on Mars reveal visible stratgraphic variations that demonstrate that the structure, lithology, and thermophysical properties of a planetary crust can vary dramatically on size scales that range from millimeters to kilometers. Given the sheer number of properties that may vary, the potential amplitude of these variations, and the unknown number and thickness of possible layers, the transport properties of the crust can potentially change by many orders of magnitude over a depth interval as small as a few millimeters. Thus, plausible permutations of crustal diffusive and thermal properties can result in intricate combinations of low- and high-permeability strata that yield substantial differences in the local distribution of ground ice.

Understanding of the subsurface distribution of ice is further complicated by the lack of knowledge regarding the nature and duration of processes involved in the geologic evolution of the local crust, among the most important of which are geographic and temporal variations in crustal heat flow. Measurements of continental heat flow on Earth (Sclater et al., 1980; Pollack et al., 1993; Stein, 1995) suggest that regional-scale (i.e., ~10^7 km^2) differences of ±50 percent can occur about the crustal mean. However, over smaller areas, the potential range of local thermal conditions on Mars is likely to be far greater. Given the scale of features and geologic diversity observed on the planet (Carr, 1996), as well as the measured heat flow of similar environments on Earth (e.g., Morgan et al., 1977; Sclater et al., 1980; Pollack et al., 1993; Stein, 1995; Dragoni et al., 2002), the thermal structure of the martian crust may vary from nearly isothermal conditions in the thickest regions of ancient highland crust to gradients of as much as 1 to 10 K/m in the vicinity of recent magmatic, volcanic, or hydrothermal activity.

This review suggests that although predictions of subsurface volatile distribution based on theoretical modeling and surface thermal observations can be of value in understanding potential global and regional-scale behavior, their use at the local scale is likely to provide little insight much below the depth that experiences daily temperature variations (~0.2 to 0.5 m), regardless of the precision and resolution to which the present thermal and radiative properties of the surface may be known. This conclusion applies not only to understanding of the distribution of ground ice, but also to virtually every other aspect of the local volatile and physical characteristics of the crust.

Geomorphic Analysis

Another major approach that has been used to infer the distribution of subsurface H_2O is geomorphic analysis, in which the size, density, and geographic distribution of landforms, attributed to the presence of subsurface liquid water or ice, have been used to infer local crustal properties. However, as a potential indicator of the present three-dimensional distribution and state of subsurface water, geomorphic analysis has two principal shortcomings: (1) its interpretations are generally not unique, and (2) the information it conveys about the potential nature and distribution of subsurface H_2O may be millions to billions of years old. These deficiencies seriously limit the utility of geomorphic indicators as reliable and quantitative guides to the present location of liquid water or ground ice, although they may provide a useful guide for assessing where more definitive investigations should be directed. Appendix F illustrates such difficulties in the context of geomorphic interpretations of martian gullies.

Geophysical Approaches

It is clear that theoretical and geomorphic approaches to assessing the distribution and state of subsurface water on Mars face many challenges. Terrestrial experience suggests that geophysical techniques may be better suited to the task. A number of such techniques are considered here.

Mars Odyssey Gamma-Ray Spectrometer

The first application of a geophysical technique to the search for subsurface water on Mars has been the GRS experiment onboard the Mars Odyssey spacecraft. The data (see Figure 4.2) indicate that, at mid- to high latitudes, the top meter of the soil is hydrogen-rich, suggesting a volumetric water ice content of as much as ~50 to 75 percent (Boynton et al., 2002; Mitrofanov et al., 2003; Feldman et al., 2003). At these latitudes, mean annual

surface temperatures fall below the ~196 K frost point temperature of atmospheric H_2O (Farmer and Doms, 1979). As a result, the level of confidence in the association of abundant hydrogen with near-surface ground ice is high.

However, for the lower abundances of hydrogen detected at equatorial and midlatitudes (where mean annual temperatures exceed the frost point), the association with ground ice is less clear. For example, at the Viking Lander 1 site (22.5°N, 48°W), which lies within the equatorial zone of reduced hydrogen abundance, data obtained by the spacecraft's GCMS instrument suggest that the soil contains ~0.1 to 1 percent by weight chemically bound H_2O (Biemann et al., 1977), most likely in the form of adsorbed water and hydrated minerals. Thermodynamically, ice at these latitudes is unstable if it is in diffusive equilibrium with the atmosphere, a condition that is likely to characterize much of the equatorial regolith, at least down to the half-meter depth sensed by the GRS instrument. However, the presence of near-surface ground ice at these latitudes cannot be ruled out. Geographic and stratigraphic variations in regolith thermal and diffusive properties, as well as the potential for subsurface replenishment, can allow ice to survive in disequilibrium with the atmosphere, at shallow depth, for billions of years—even at the warmest locations on the planet (Smoluchowski, 1968; Clifford, 1998).

Although the data acquired by the GRS suite of instruments indicate a marked contrast in the abundance of near-surface hydrogen between low and high latitudes, the magnitude and uniformity of this appearance may be deceiving. With a best integrated instrument surface spatial resolution of the order of ~ 3×10^5 km^2, the indicated abundances of hydrogen in Figure 4.2 are averaged over areas that are many orders of magnitude larger than the scale of heterogeneity in physical, thermal, and compositional properties exhibited by the planet's surface. Thus, at high latitudes, there may be many localized areas where the concentration of near-surface ground ice is actually quite small (i.e., <10 percent), interspersed with larger regions where the volumetric ice content approaches 90 to 100 percent. The same may be true of ground ice at lower latitudes, although there the relative abundance of ice-rich to ice-poor regions could be reversed. The GRS data show low-latitude regions with bulk water abundances that approach maximum values of ~10 percent, but the forms of that water, be it chemically bound, adsorbed, or ice, or spatially varying combinations of all three, have not yet been determined.

For mission planners attempting to locate or avoid ice-rich regions, the dilemma is that, given the currently available data, one cannot assess the characteristic scale and magnitude of local heterogeneity with respect to the near-surface distribution of ground ice. A second problem is that, although the GRS investigations provide a wealth of data on the large-scale distribution of hydrogen within the top meter of the regolith, they provide no insights regarding the presence of water at greater depths. Both higher spatial resolution and probing of greater depths are needed. One approach to obtain these data is through active radar sounding.

Radar Sounding of the Martian Subsurface

On Earth, radar sounding from surface, aerial, and orbital platforms has been used successfully to investigate a variety of terrains and subsurface environments, including hot arid deserts (McCauley et al., 1982, 1986; Daniels et al., 2003), Arctic permafrost (Annan and Davis, 1976; Arcone et al., 1998), glaciers (Arcone et al., 2000; Degenhardt and Giardino, 2003), and polar ice sheets (Robin et al., 1969, 1977; Siegert, 2000). Radar sounding was also successfully conducted from lunar orbit by Apollo 17 (Phillips et al., 1973), although in comparison with terrestrial studies, the interpretation of those data have proved far more challenging, due in large part to the lack of available ground truth (Peeples et al., 1978; Sharpton and Head, 1982).

Terrestrial experience has demonstrated that ground-based geophysical exploration, using a combination of different techniques, is well suited for the search for subsurface water on Mars (Stoker, 1998; Beaty et al., 2000; Clifford et al., 2001). But a strategy to search for subsurface water on Mars by proceeding directly to the use of ground-based instruments has the drawback that such instruments provide no global context. For this reason, the European Space Agency selected the Mars Advanced Radar for Subsurface and Ionospheric Sounding (MARSIS) among the instruments to fly on the Mars Express spacecraft, which successfully entered Mars orbit on December 25, 2003.[11] One of the primary goals of the MARSIS investigation is to conduct a preliminary global reconnaissance

[11]For further background on MARSIS and Mars Express, see <www.esa.int/SPECIALS/Mars_Express/>.

for subsurface water. The results will help target more capable and higher-resolution surface investigations in the future. A technical discussion of the mode of operation of MARSIS is provided in Box 4.1.

The depth achieved by a radar sounder is maximized at low frequencies. For this reason, MARSIS was designed with a low operational frequency range of 0.5 to 5 MHz, with the lower limit imposed by the potential for interference from the ionosphere. At these frequencies, MARSIS will have a vertical subsurface resolution of approximately 100 m and a maximum projected penetration depth (under ideal sounding conditions) of as much as several kilometers. However, propagation losses due to the electrical and magnetic properties of the crust are likely to have a significant impact on the sounding performance of MARSIS (Beaty et al., 2001a; Heggy et al., 2001, 2003; Grimm, 2003). Specifically, the transmission, reflection, and loss experienced by a radar pulse depend on the electromagnetic properties and volumetric contribution of the regolith's various components, as modified by its porosity and the degree of pore saturation by air, ice, and liquid water or brine (Olhoeft, 1998; Leuschen et al., 2003; Heggy et al., 2003; Grimm, 2003). Transmission is maximized in high-porosity, low-iron-content lithic materials whose pores are saturated with nonconducting air or ice, while the strength of a reflection from the

BOX 4.1
MARSIS: Operational Description of an Orbital Radar Sounder

MARSIS is a multifrequency, coherent-pulse, synthetic aperture radar sounder whose operation resembles that of a more traditional ground-penetrating radar (GPR). MARSIS operates by emitting a short electromagnetic pulse whose transmitted power is reduced at the planet's surface by $1/R^2$, where R is the sounder's altitude above the ground. When this pulse reaches the boundary between two materials of differing dielectric properties (such as the atmosphere and the ground), a portion of the incident energy is reflected back to the sounder (once again, undergoing a $1/R^2$ loss)—while the remainder continues to propagate into the subsurface, where it may suffer additional losses due to scatter by embedded objects and absorption by the host material. As successive dielectric interfaces are encountered, the signal experiences additional reflections and losses by absorption (affecting both the transmitted and the reflected portions of the signal). As the reflected signals are received by the orbital sounder, the depth of the corresponding interface can be determined by measuring the time delay between transmission and reception, as long as the returned signal is still detectable above the background noise.

Given the high dielectric contrast between liquid water and ice or rock, it has been predicted that MARSIS will be able to detect a liquid water interface over a depth range of 0.3 to 5 km with a signal >3 dB above the noise, assuming a sharp transitional boundary, a saturated porosity >10 percent, and favorable conditions of surface roughness (rms slopes <0.5°, which characterize ~20 percent of the planet). Under the most favorable conditions of surface roughness, target geometry, and rock composition, MARSIS may also be capable of detecting the much smaller dielectric contrast between massive lenses of segregated ice (where the saturated porosity is near 100 percent) and ice-free or ice-saturated frozen ground (Beaty et al., 2001a). The unambiguous identification of crustal H_2O may prove difficult or impossible using orbital sounding alone (Beaty et al., 2001a,b). But the geometry and contrast associated with the volatile targets of greatest scientific interest, such as liquid water and massive ground ice (MEPAG, 2004), are expected to be sufficient so that, under favorable observing conditions (and with the additional interpretive context provided by the analysis of other remote sensing data), probable occurrences should be identifiable. In this way, it is hoped that MARSIS will provide a first look at the lithology, structure, and volatile stratigraphy of the martian subsurface.[1]

[1]Although MARSIS was originally scheduled to begin operation in May 2004, concerns regarding potential damage to the spacecraft from the deployment of the radar antenna resulted in a 1-year delay in both antenna deployment and data collection. In May 2005, the antenna system was successfully deployed, and following a brief commissioning period, MARSIS began acquiring data in July 2005, but no results were available at the time this report went to press.

interface between two materials is aided by a high dielectric contrast and sharp boundary transition. In contrast, the presence of abundant iron (particularly as magnetic phases), liquid water, and especially electrically conductive brines can reduce a radar sounder's performance by as much as several orders of magnitude, even at low frequencies. Given the range of composition, physical and electromagnetic properties, and local environmental conditions found on Earth (many of which may also be present on Mars), the actual sounding depth achieved by MARSIS is likely to vary enormously from essentially no penetration in some areas to as much as several kilometers in others.

The combination of the low frequencies used by MARSIS and interference arising from off-nadir reflections creates a potential blind zone that may prohibit MARSIS from resolving variations in dielectric contrast at depths shallower than several hundred meters. In 2005, NASA plans to launch the Mars Reconnaissance Orbiter (MRO), which includes the shallow radar (SHARAD) instrument (Seu et al., 2004). As with MARSIS, the primary mission of SHARAD is to search for dielectric evidence of subsurface water and ice, but at shallower depth and higher resolution—a capability derived from SHARAD's higher (20 MHz) operating frequency and 10-MHz bandwidth. Additional radar instruments either are planned or have been proposed, as part of both surface- and orbiter-based missions to Mars in 2009 and beyond.

MEASUREMENTS NEEDED TO IDENTIFY SPECIAL REGIONS

For mission planners and scientists who wish to identify special regions in order to either avoid or explore them, there are three principal concerns: (1) What types of measurements are necessary to identify special regions? And to what resolution? (2) What instruments are capable of making these measurements? And on what platforms are they most effectively employed? (3) What are the ambiguities associated with this identification? And how can they be reduced?

Identifying the distribution of special regions on Mars requires understanding the present three-dimensional distribution and state of water in the subsurface. As noted above, orbital and surface geophysical investigations appear to offer the best approach for acquiring this knowledge—yet, no single technique, or even combination of techniques, is capable of providing a complete understanding. To make the greatest progress in assessing the distribution and state of subsurface water it is necessary to identify those investigations that maximize knowledge of the location of the most sensitive environments.

The chief immediate concern in locating special regions is to identify where liquid water and massive ground ice are present within the top ~25 m of the regolith, the maximum depth to which currently envisioned investigations by robotic spacecraft will be capable of reaching over the next decade. In addition, because variations in regolith thermal and diffusive properties—capable of preserving liquid water or ice at shallow depth—can occur at small scales, an understanding of the distribution and state of subsurface H_2O at as high a resolution as practically possible (ideally, at a scale equivalent to that defined by the operational activities and landing accuracy of the investigating robotic spacecraft) would provide the greatest level of assurance in researchers' ability to access or avoid special regions.

Terrestrial experience has demonstrated that the accurate identification of subsurface groundwater and ground ice is likely to require the application of multiple geophysical techniques (Stoker, 1988)—investigations that are most effectively conducted on (or in close proximity to) the planet's surface. But the relative fraction of the martian surface that can be investigated by a single lander, rover, or even aerial platform is small, requiring a prohibitively large number of spacecraft to conduct a global survey. Therefore, local investigations are most effectively employed following the completion of an initial global reconnaissance to identify regions of interest, a survey that is most easily accomplished from orbit.

Orbital Measurements

The electrical and magnetic properties of the crust are particularly diagnostic of the presence of conducting liquid water and brine. However, the much smaller contrast between the dielectric properties of rock and ice makes the unambiguous identification of ice much more difficult, except where it may be present in the form of massive

segregated deposits. Thus, geophysical investigations can provide valuable insights regarding the occurrence of two of the most sensitive special-region environments, those where liquid water or massive ground ice are present, but may be unable to exclude the potential presence of ice-rich frozen ground without the assistance of invasive surface investigations using penetrators or shallow drills.

MARSIS and SHARAD were not optimized to collect data on the near-surface (~1 to 100 m) range of greatest immediate concern in the identification of special regions (see Box 4.1). Because of its low operating frequencies (1 to 5 MHz), MARSIS may have an effective blind zone that extends from the surface down to a depth of ~300 m, a consequence of the instrument's inability to distinguish between off-nadir reflections due to local surface clutter and reflections from the shallow subsurface. The higher operational frequencies of SHARAD (15 to 25 MHz) could reduce this blind zone to ~50 m (Beaty et al., 2001b), but that would still be a depth exceeding that of greatest concern for the identification of special regions. The actual capabilities of these instruments in the martian environment will soon be known, allowing such expectations to be reassessed in the context of actual data.

The depth of the blind zone should shrink dramatically at higher frequencies, which also significantly improve the horizontal and vertical resolution of the sounder. The principal disadvantage of higher frequencies is a reduction in maximum penetration depth. This penalty can be offset by operating the sounder over a broad range of frequencies that provide high spatial resolution near the surface and coarser resolution at depth. For depths of 0 to 25 m, a frequency range of 100 MHz to 1 GHz appears ideal, particularly if such a sounder were complemented with a synthetic aperture imaging radar. Such a combination would be expected to detect any liquid water or brine present in the top 25 m of the subsurface, provided its volumetric fraction was more than a few percent, and would also offer a significant enhancement in the ability to identify deposits of massive ice in the near-subsurface (such as a fossil snowpack or the ponded frozen discharge of the gullies, outflow channels, or a relic of a former sea or ocean).

As described earlier, gamma ray and neutron spectrometry from orbit can provide strong quantitative constraints on the distribution of hydrogen in the near-surface environment. The chief limitation of this technique as it has been applied to date, is its low spatial resolution. This limitation could be improved in future experiments by using higher-resolution collimated sensors as with the Lunar Exploration Neutron Detector (LEND) experiment to fly on the 2008 Lunar Reconnaissance Orbiter, or by measurements from lower-altitude airborne platforms.

Surface Measurements

Global orbiter measurements intended to identify special regions with good spatial resolution will require ground truth measurements of the electromagnetic, physical, and compositional properties of the top meter of the regolith at multiple locations around the planet. The need to obtain ground truth at multiple sites is driven by both the natural heterogeneity of the surface and the various factors that may contribute to the origin of subsurface reflections and signal loss. The acquisition of such data would significantly reduce the level of ambiguity associated with the interpretation of orbital radar data—especially if combined with ground-based geophysical investigations at these same locations. Possible candidates include the addition of magnetotelluric and permitivity instruments, active and passive seismometers, and ground-penetrating radar (GPR). Such an approach would greatly improve the ability to discriminate between lithologic and volatile units.

For platforms such as landers, rovers, and penetrators that can directly access the near-surface region, a variety of measurements can be deployed that could shed light on the existence of and/or potential for special environments. Examples include measurements of physical parameters such as temperature, pressure, soil thermal and adsorptive properties, electromagnetic properties, and gas and liquid permeability; measurements of gas composition, with particular emphasis on the partial pressure of atmospheric water vapor; measurements of the soil and rock chemistry, including the abundances of potential solutes in brines, and parameters such as pH and eH; and direct time-dependent measurements of the abundance of water in its various forms. It should be noted that most of the above measurements have yet to be successfully acquired at any location on Mars.

SPACECRAFT ACCESS AND SPECIAL REGIONS

In the wake of a best-effort orbital reconnaissance for special regions, there will always be some uncertainty regarding the designation of any region as nonspecial—either because of the presence of dispersed ice or because of the concentration of liquid water or massive ice at spatial scales that are much smaller than can be surveyed from orbit. The characterization of special regions should evolve with improved technological capabilities for exploring the near-surface and subsurface of Mars. Further, the degree to which robotic spacecraft could accidentally or intentionally access the subsurface (e.g., as a consequence of mission failure that results in a spacecraft's high-velocity impact into the surface) or by purposeful intent (e.g., by trenching with a robotic arm, drilling into the subsurface, or by the emplacement of a penetrator, or, in the case of ice, by melting down with a cryobot) affects the depth and resolution of understanding about the subsurface regolith that may be required to prevent contamination and could eventually necessitate an understanding of depths greater than 25 m. For example, if deep-drilling investigations of the crust were anticipated, then a knowledge of the distribution of liquid water and massive ground ice at even greater depth (e.g., ~100 to 1000 m) could be desirable. In Chapter 8, the committee presents its conclusions regarding planetary protection requirements that should be followed by spacecraft exploring regions that cannot confidently be defined as nonspecial.

Between 2005 and 2020, it appears unlikely that such activities will access depths much greater than ~25 m. However, efforts are already under way to identify and develop techniques capable of accessing depths ranging from many tens to thousands of meters beneath the surface (Blacic et al., 2000) to conduct astrobiological research.

SUMMARY

By the end of the Viking mission, Mars was broadly viewed as a very inhospitable world (e.g., Horowitz, 1986). The data available then suggested that the planet had little surface water, and the low atmospheric pressure, surface temperature, and high UV flux appeared extremely hostile to even the hardiest terrestrial organisms known. Knowledge of Mars has since progressed enormously, as has knowledge of the limits of terrestrial life (see Chapter 5). Although the surface of Mars continues to appear inhospitable to Earth life (e.g., Schuerger et al., 2003), the inventory of martian water in all its forms seems substantial.

The martian surface environment is salt-rich, and plausible combinations of salts could result in the transient presence of cold liquid water environments in the near-surface regolith on a diurnal and seasonal basis over much of the planet. Indeed, at low latitudes, it is possible that brines may persist continuously at all depths within the crust. Even in the absence of salts, summertime temperatures at low latitudes can still exceed the freezing point for up to several hours a day within the top 1 to 2 cm. More persistent occurrences of liquid water may be found in association with localized volcanic and magmatic activity and at depths of several kilometers or more, where geothermal heating may elevate crustal temperatures above the freezing point. It is not known whether geothermal hot spots may exist in the near-subsurface at some locations. Water in the form of ice is more widely distributed, appearing to be the dominant constituent of the polar-layered deposits and the near-surface regolith at mid- to high latitudes. Near-surface ice may also be present in smaller amounts at equatorial latitudes and is likely present globally at depth.

Currently, there are no data to distinguish between areas that are water-rich versus water-poor at spatial resolution better than ~3×10^5 km^2. Yet, recent observations show that Mars exhibits significant horizontal and spatial diversity on spatial scales of kilometers to centimeters. This fact makes it difficult currently to designate with confidence any specific region of the planet as special or nonspecial. Coming to grips with the many issues relating to Mars's current habitability will require a continued broad and sustained program of interdisciplinary scientific exploration of Mars, and also measurements specifically targeted to locating special regions, especially to identify where liquid water and massive ground ice are present within the near-surface. In addition, because variation in regolith thermal and diffusive properties—capable of preserving liquid water or ice at shallow depth—can occur at small scales, an understanding of the distribution and state of subsurface H_2O at a resolution and at a scale equivalent to that defined by spacecraft operational activities and landing accuracy would improve confidence in the ability to access or avoid special regions.

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5

Expanding Our Knowledge of the Limits of Life on Earth

Strategies for minimizing the risk of forward contamination in solar system exploration rely on knowledge about the kinds and numbers of microorganisms carried by a spacecraft. Such descriptions of microbial population structure provide a basis to estimate the likelihood of survival during spaceflight and to model the probability of subsequent growth in an extraterrestrial environment. Implicit in these models is knowing the range of environmental parameters compatible with growth of microbial populations in both nominal and extreme environments[1] on Earth. In recent years, the combination of traditional microbiology and new technological advances has identified organisms that can survive and grow under conditions previously thought hostile to life, including extreme temperatures, radiation, high ionic strength, and desiccation. Some of these conditions are similar to those in environments that occur elsewhere in the solar system where terrestrial spacecraft have visited or will visit in the near future. In particular, some of these conditions resemble those likely to occur on Mars, as described in Chapter 4. The current chapter summarizes the changing perspective of microbial life in extreme environments and how technology enables the detection and enumeration of different kinds of organisms in microbial populations. The chapter then explores the limitations, within the context of existing knowledge, to estimating the probability that Earth organisms could grow on Mars.

MODERN VIEWS OF MICROBIAL DIVERSITY

Detection, characterization, and enumeration of single-cell organisms necessarily depend on optical imaging, cultivation, and biochemical or molecular technology. The invention of the microscope in the 1600s was a revolutionary advance that revealed a never-before-seen world of single-cell organisms. Over the ensuing 300 years, biologists relied on microscopy, nutrients required for growth, and biochemical characterization to describe microbial diversity. Differences in morphology, staining characteristics, metabolic capabilities, and physiological properties defined boundaries between different kinds of microorganisms.[2] Serial dilution assays[3] and colony

[1]"Extreme environment" is an anthropocentric term that refers to physical and chemical conditions outside what was once perceived to be hospitable to life. It is now clear that such conditions can be endured by a wide range of microorganisms.

[2]The macroorganismal definition of a "species" is a population of individuals that can interbreed under natural conditions and produce fertile offspring, and are reproductively isolated from other populations. This definition does not apply to archaea, bacteria, and most protists, owing to their asexual mode of reproduction and lack of true homologous recombination. Early taxonomic treatments of microorganisms used

formation by individual cells on agar growth medium provided estimates of microbial population sizes. Twenty years ago, microbiologists had described only 5,000 kinds of bacteria and archaea, and biodiversity studies largely ignored the contribution of single-cell organisms to Earth's global biomass. These modest assessments of microbial diversity and population size were not consistent with a ~3.5 billion-year evolutionary history, during which single-cell organisms have developed an enormous metabolic repertoire to cope with Earth's dynamic environment. Such underestimates of microbial diversity reflect how difficult it is to identify morphological and biochemical characteristics (phenotypic traits) that are uniquely shared between closely related organisms, as opposed to common ancestral features that persist over the largest of evolutionary timescales.

Discoveries over the last 20 years of microbial life in habitats previously thought to be devoid of life (e.g., hot springs, deep-sea vents, solid ice, subglacial environments), and the ability to detect microorganisms without requiring their cultivation in the laboratory, have greatly expanded our knowledge of habitat range and numbers of microbes in Earth's biosphere. For example, fluorescent DNA staining of bacterial cells and epifluorescence microscopy revealed that microbial numbers in aquatic environments were 100 to 1,000 times greater than estimates based on cultivation techniques.[4] Contemporary culture-independent surveys demonstrate that microbes may be the dominant biomass on Earth (Whitman et al., 1998), and microbes account for most of the genetic and metabolic diversity (Pace, 1997). The number of different kinds of microbes remains unknown, but the use of modern molecular technology indicates that traditional estimates of 5,000 kinds of microbes are low by several orders of magnitude. In addition to high diversity, the number of archaean and bacterial cells on Earth (see Table 5.1) exceeds 3.6×10^{30}, while viral and bacteriophage titers are several orders of magnitude higher. These microbial abundances yield a total carbon content that is 50 percent or more of the estimated carbon in all terrestrial plants (Whitman et al., 1998).

MODERN TECHNOLOGY AND MICROBIAL ECOLOGY

The direct interrogation of microbial genomes offers a powerful, culture-independent method for exploring microbial diversity. Every species has a unique collection of gene sequences that make up its genome, and each species can have a very different complement of genes. The collection of gene sequences in a genome specifies the constellation of proteins that orchestrate cellular biochemistry. The aggregate biochemical activity defines the character or phenotype of a particular kind of organism. The DNA sequence of a gene can serve as a proxy for species identification if that gene is present and conserved by evolution in both the unknown and a reference set of well-characterized organisms. The comparison of gene sequences that share a common evolutionary history allows the reconstruction of evolutionary history or phylogeny. When an organism of unknown taxonomic affinity is included in such analyses, computer algorithms can infer its phylogenetic placement in the context of a large molecular database of well-characterized lineages.

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comparison of morphological and physiological traits to differentiate between microbial species. Molecular techniques including DNA:DNA hybridization, determination of GC content of the genome (percentage of the nucleotides containing guanine and cytosine), and phylogenetic inferences based on comparisons of small-subunit ribosomal RNA gene sequences provided more powerful tools for differentiating among microbes. Yet an attempt to define species boundaries according to a defined number of nucleotide differences between homologous genes is arbitrary and in some cases fails to resolve different populations in situ (e.g., Ward et al., 1990).

[3]A serial dilution assay is a common method for enumerating numbers of bacteria by counting the number of colonies formed by aliquots of sample dilutions spread onto an agar surface. A small known amount of sample is mixed with a sterile diluent solution (water or liquid media), and a 0.1- to 1.0-ml aliquot of the mixed diluent (containing the initial sample) is spread on an agar surface. In a similar way, successive dilutions are prepared from each diluent and 0.1- to 1.0-ml aliquots are spread onto an agar plate. After counting the number of bacteria colonies that grow on each plate, it is possible to backcalculate, using the "dilution factor" (the number of times that bacteria sample was diluted with the diluent solution), the number of bacteria in the original sample.

[4]Epifluorescent microscopy relies on the excitation of susceptible molecules in a sample with short-wavelength, high-energy light and observation of the emitted lower-energy light (fluorescence). Susceptible molecules include autofluorescent compounds such as chlorophyll and F420 (an enzyme cofactor found in methanogens) or fluorophores that bind to specific cellular compounds. Epifluorescent microscopy enables researchers to observe and detect cellular components that were not visible with conventional light microscopy, and it is sensitive enough to detect a single molecule.

TABLE 5.1 Microbial Diversity: Selected Terminology

	Description
Type Diversity	
Prokaryotes	A general term that encompasses bacteria and archaea, microbial cells that lack a nuclear membrane surrounding their chromosomal DNA, a cytoskeletal matrix, and other membrane-bounded organelles such as mitochondria and chloroplasts
Bacteria	One of the three known domains of life: microbial cells lacking nuclear bound chromosomes with predominantly diacyl glycerol diester membrane lipids
Archaea	One of the three known domains of life: microbial cells lacking nuclear bound chromosomes with predominantly isoprenoid glycerol diethers or diglyceral tetraether membrane lipids
Eukarya	One of the three known domains of life with nuclear bound chromosomes, cytoskelatal organizing matrices, predominantly glycerol fatty acyl diester membrane lipids, and other membrane-bounded organelles such as mitochondria and chloroplasts
Psychrophile	Capable of growth at low temperatures, with an optimal growth temperature below 15°C
Psychrotroph	Capable of growth at low temperatures, with an optimal growth temperature greater than 15°C
Mesophile	Generally defined by optimal temperature for growth, usually between 25°C and 40°C, but often capable of growth from 8°C to 50°C
Thermophile	Optimal temperature for growth is greater than 45°C but not above 80°C
Hyperthermophile	Optimal temperature for growth is 80°C or above
Acidophile	Grows at pH values less than 5
Alkalophile	Grows at pH values greater than 9
Neutrophile	Grows with optimal rates near pH 7
Halophile	Requires high salt concentrations (>2.5 M) for growth
Xerophile	Capable of growing under conditions of low water activity (effective water content)
Barophile	Obligate barophiles are unable to grow at 1 atmosphere of pressure; barotolerant bacteria grow at 1 atmosphere and higher pressures; all barophiles grow optimally under high pressure
Physiological Diversity	
Aerobe	Capable of using oxygen as terminal electron acceptor; can tolerate levels of oxygen at or greater than 21 percent and has a strictly respiratory-type metabolism
Anaerobe	Grows only in the absence of oxygen; most have fermentative-type metabolism, but some carry out anaerobic respiration using terminal electron acceptors other than oxygen
Facultative anaerobe	Can grow aerobically or anaerobically
Microaerophile	Capable of oxygen-dependent growth at oxygen levels well below 21 percent
Autotroph	Uses carbon dioxide as its sole source of carbon
Heterotroph	Unable to use carbon dioxide as a sole source of carbon and requires one or more organic compounds
Chemoorganoheterotroph	Derives energy from chemical compounds and uses organic compounds as a reductant
Chemolithoautotroph	Relies on reduced chemical compounds as a source of energy and carbon dioxide as a source of carbon; includes hydrogen bacteria, iron bacteria, sulfur bacteria, ammonia oxidizers, nitrite oxidizers obligate methane oxidizers, carbon monoxide oxidizers
Mixotroph	Capable of growing both chemoorganoheterotrophically and chemolithoautotrophically
Oligotroph	Capable of growth on minimal media (1 to 15 μg carbon per liter)
Copiotroph	Requires nutrients at levels 100 times those of oligotrophs

SOURCE: Madigan et al. (2002).

There are a variety of methods for analyzing genes from complex, naturally occurring microbial populations. Most rely on the use of gene cloning and/or polymerase chain reaction (PCR)[5] to obtain sufficient quantities of a specific gene for DNA sequence analysis. Such culture-independent characterizations of genes that are conserved over evolutionary time have provided many insights about novel metabolic and physiological categories of microbes (Béjà et al., 2000), as well as information about their distribution in nominal and extreme environments. Analyses of ribosomal RNA (rRNA) coding regions have proven particularly informative. These genes are conserved in all known organisms because they are required for the translation of information encoded within genomic DNA into proteins. Current molecular databases contain more than 120,000 reference rRNA sequences (phylotypes) from diverse microbial forms.[6] This window on the microbial world has revealed new levels of largely unexplored microbial diversity not represented in laboratory cultures. According to these molecular studies, microbial diversity ranges from 10^5 to more than 10^7 kinds of organisms (Pace, 1997). Evidently, traditional microbiology has failed to culture more than 99.9 percent of these newly discovered phylotypes. Much of the newly discovered diversity resides within described lineages, but ongoing investigations continue to reveal deep-branching, basal lineages in all three domains of life (Lopez-Garcia et al., 2001; Moon-van der Staay et al., 2001; Edgcomb et al., 2002; Cifuentes et al., 2000; Dojka et al., 1998, 2000; Dawson and Pace, 2002). Because little is known about the physiology and survivability of many of these phylotypes, their potential impact on forward contamination from spacecraft represents a major concern for planetary protection. For example, even some organisms that do not form heat- and desiccation-resistant endospores[7] might survive spaceflight and be capable of colonizing other solar system objects. Also, the large number of undescribed phylotypes may have levels of resistance to such stress that exceed those known for microbes that have been cultured and characterized, possibly including known spore-forming organisms.

Molecular techniques also have demonstrated that microbial lineages that are not easily cultivated sometimes dominate naturally occurring microbial populations. An example of an uncultured group that has been found (by 16S rRNA clone library analysis) to represent a major fraction of the noncultured bacteria in soils, and whose members have vastly different physical and chemical characteristics, is the Acidobacterial Division. Although their function in soils and sediments remains unknown, these bacteria are thought to play important roles in bacterial community function (Felske et al., 2000). Another example is the marine SAR 11 lineage first identified in the early 1990s from samples collected in the Sargasso Sea. These microorganisms were identified qualitatively via gene cloning as belonging to a major group of uncultured bacterioplankton[8] in the sea. Yet, scientists have lacked good quantitative information about how this specific group of bacteria contributed to the total oceanic bacterial pool. The use of molecular techniques in combination with microscopy has now shown that SAR 11 makes up as much as 50 percent of the total surface microbial community (from 0 to 140 m below the surface) and 25 percent of the rest of the water column down to the bottom of the sea (Morris et al., 2002; Rappe et al., 2002).

ORGANISMS AT THE LIMITS OF LIFE

In recent years, spectacular discoveries have altered researchers' ideas about requirements for microbial growth and the ability of microbes to thrive under seemingly inhospitable environmental conditions. Thermophilic

[5]PCR is a molecular technique that can generate many copies of a gene that lies between two regions of known sequence in a genome. For PCR experiments, short oligonucleotide primer pairs that are complementary to conserved domains separated by 1,000 to 2,000 base pairs anneal to single-stranded templates and initiate DNA synthesis with heat-stable DNA polymerase. After completion of the chain-elongation cycle, treatment with heat (90°C) dissociates the new strands to form new single-stranded templates for subsequent rounds of primer re-annealing and DNA synthesis. Each cycle of heating, renaturation of primers with new templates, and DNA synthesis (chain elongation) doubles the DNA sequence between the conserved primers, providing an exponential amplification of the DNA over many cycles. This process is illustrated in Box 6.1.

[6]See, for example, <http://rdp.cme.msu.edu/html/>.

[7]Endospores (or simply spores) are small, usually single-celled reproductive bodies produced by certain bacteria in response to adverse environmental conditions. They are highly resistant to desiccation and heat, and if viable, are capable of growing into a new organism under favorable conditions. See also footnote 2, Chapter 1.

[8]"Bacterioplankton" refers to the fraction of life in the water column of the oceans (and lakes) that includes bacteria and archaea.

microorganisms can thrive at 121°C (Kashefi and Lovely, 2003), whereas cold-adapted lineages (e.g., psychrophiles) are active in icy systems below –15°C (Rivkina et al., 2000; Carpenter et al., 2000; Christner, 2002; Thomas and Dieckmann, 2002). Microbes populate highly acidic (pH ~0.0) and alkaline (pH >12) environments. Some bacteria tolerate very high levels of radiation and desiccating conditions. Chemosynthetic microbial communities near deep-sea hydrothermal vents (Jannasch and Mottl, 1985), bacteria in deep marine sediments (Bale et al., 1997; Parkes et al., 2000), and subglacial microorganisms (Sharp et al., 1999) thrive without direct requirements for solar input. Some subsurface ecosystems may be entirely independent of surface conditions (Chapelle et al., 2002; Priscu et al., 1999b). Bacteria and archaea occupy every imaginable niche and sometimes occur in settings that seem totally incompatible with biological activity. Some microbes survive such conditions by entering into dormant or resting stages (e.g., bacterial spores), whereas extremophiles have adapted physiologies that nurture growth rather than dormancy under these harsh conditions.

Extremophiles are organisms that thrive in what, for most life forms, are intolerably hostile environments.[9] The majority of known extremophiles are representatives of the domains Archaea and Bacteria, although certain protists[10] have also been found to thrive in these environments. Extremophiles are classified according to the conditions in which they exist, for example as thermophiles (hot), psychrophiles (cold), halophiles (salty), acidophiles (acidic), alkaliphiles (basic), and barophiles (under pressure) (see Table 5.1). These categories are not mutually exclusive; for example, some acidophiles are also thermophiles.

Extremotrophs represent another category of organisms often found in hostile environments. Extremotrophs tolerate the conditions in question but are just barely able to survive them. The only obligate environmental growth constraint for these and all types of microorganisms is a requirement for liquid water. The discovery of extremophiles and extremotrophs points out the extraordinary adaptability of microbial life on Earth and raises the prospect of finding at least microbial life elsewhere in the solar system.

LIFE IN EXTREME ENVIRONMENTS

Cold Environments on Earth and Mars

Earth's biosphere is cold. Fourteen percent of the biosphere is polar and 90 percent, by volume, of the world's oceans are below 5°C. More than 70 percent of Earth's freshwater occurs as ice, and a large portion of the soil ecosystem (~20 percent) exists as permafrost (Priscu and Christner, 2004). Microorganisms in sea ice were noted by early sailors and first studied as a scientific curiosity by Bunt (1964). Despite the global significance of sea ice, which accounts for about 67 percent of Earth's ice cover, only recently has it been explored for novel microorganisms (Thomas and Dieckmann, 2002; Junge et al., 2002, 2004). Studies of microbial diversity in polar oceans have shown, in addition to psychrophilic bacteria (Wells and Deming, 2003; Huston et al., 2004), a predominance of archaea in the subgroup Crenarchaeota (DeLong et al., 1994). Before that discovery, crenarchaeota were thought to be confined to thermal systems. Detection of microbial life in cold (–5°C) and saline (>5 times saltier than seawater) Antarctic lakes (Priscu et al., 1999a; Takacs and Priscu, 1998; Doran et al., 2002), permanent lake ice (Priscu et al., 1998; Fritsen and Priscu, 1998; Psenner et al., 1999), glacial ice (Christner et al., 2000, 2001; Smith et al., 2004), and polar snow (Carpenter et al., 2000) extends the known boundaries of our biosphere. The recent description of potential bacterial life in Lake Vostok (Priscu et al., 1999b; Karl et al., 1999; Siegert et al., 2001; Bulat et al., 2004) and the discovery of at least 140 other Antarctic subglacial lakes (Priscu et al., 2003) further extend the recognized environmental limits for life on Earth. Yet, the spatial and temporal records for icy systems on Earth are sketchy, and relatively little is known about the psychrophilic or psychrotolerant microorganisms that inhabit them.

Because of the near- or below-freezing temperatures of the martian surface, most microbes will likely exhibit long generation times (the time required to double the number of organisms in a microbial population) that range

[9]The term "extremophile" is anthropocentric because the hostile environment (from a human point of view) is quite normal to the organism itself. See also footnote 1 in this chapter.

[10]Protists are single-celled eukaryotes.

from several to many years. Microorganisms capable of growth at subfreezing temperatures with generation times shorter than 1 to 2 years would likely pose the greatest challenge to planetary protection during the next 50 to 100 years.[11] There are claims of microbial growth between –17°C and –20°C (e.g., Junge et al., 2004; Carpenter et al., 2000; Priscu and Christner, 2004; Christner, 2002), although their rates are slow owing to thermodynamic constraints (Price and Sowers, 2004).

Under ideal laboratory conditions, psychrophiles capable of growth at low temperature often exhibit generation times that range from hours to weeks (e.g., Ingraham, 1958; Innis, 1975; Ward and Priscu, 1997). Despite these claims, confusion exists about phychrophilic growth at low temperatures: Do psychrophiles, mesophiles, and thermophiles grow at equivalent rates at their respective optimum growth temperatures? This is not a simple question to answer because there is a large variation in the growth rates of different organisms within each group, as well as a large variation in growth rates for an individual microbe, depending on growth parameters such as oxygen supply, nutrient type, nutrient concentration, and pH. Many psychrophiles have generation times in rich liquid culture of a few hours at or close to 0°C (Russell and Hamamoto, 1998). Such values are comparable to those for environmental isolates of mesophiles, although much shorter times have been reported for the latter. It is difficult to extrapolate experimentally measured generation times to growth rates in natural settings, but evidence so far indicates that growth rates in nature are usually lower than those in enriched media (e.g., Ward and Priscu, 1997).

Because of short durations of liquid water[12] and the likely absence of organic substrates due to the near-surface oxidizing environment, the growth of psychrophiles in the martian near-surface is likely to be even slower than the growth rates measured in many Earth environments. Chapter 4 describes what is currently known about the distribution and state of water on Mars. Liquid water may be present for short durations near the martian surface, although scientists cannot rule out the longer-term occurrence of liquid water near the surface (e.g., if active hydrothermal systems exist) or, especially, deeper (e.g., at depths where temperatures are always too high for freezing to occur). At low latitudes, the temperature of the top few centimeters of the regolith may exceed 0°C for a few hours of the diurnal cycle, and liquid water may exist at higher latitudes that have low albedo, poorly conductive soils, or large equatorial-facing slopes. Although liquid water may be present for short periods of time in a variety of locations, organisms that experience temperature fluctuations between 0°C and <–20°C must mount stress responses to the damage caused by eutectic freezing and thawing. Under these conditions, there is little time for both recovery and growth over a diurnal cycle where liquid water is present for at most a few hours each day. The generation time for bacteria exposed to temporal freeze-thaw cycles in the permanent ice covers of Antarctic lakes has been estimated to average 2.5 years and can be as long as 9 to 10 years, depending on the annual duration of liquid water (Fritsen and Priscu, 1998).

Increases in cell numbers over time can provide a measure of bacterial growth, but organisms may be metabolically active and increase their total biomass through enlarged cells rather than an increase in cell number. Temperatures below those required for optimal growth generally constrain maximal rates of division but not necessarily productivity (Knoblauch and Jorgensen, 1999).

Recent models predicting that active cells can survive over hundreds of thousands of years at –40°C (Price and Sowers, 2004) have implications for planetary protection policies, if such policies were to span not some decades-

[11]As a quantitative example, consider a single microorganism inoculated into a martian habitable zone of transient liquid water over a square kilometer to a centimeter depth. Over a 50-year period, this inoculation would produce contamination levels of fewer than 100 microbial cells per milliliter (the sensitivity threshold for many life-detection technologies) if the daughter cells were distributed throughout this zone and if the average generation time (the time required between cell divisions) was more than 1.2 years. If the inoculum consisted of 1,000 initial cells, the corresponding required generation time to reach 100 cells per milliliter in 50 years would be 1.6 years. Organisms with generation times shorter, or even much shorter, than a year under martian conditions—should such combinations of terrestrial microorganisms and martian habitats exist—could pose a more substantial threat to planetary protection over a period of decades. It is such potential organisms and environments, therefore, that are of the greatest concern.

[12]Short duration, in this context, means that a bulk liquid water environment exists for a period shorter than a microorganism's doubling time. The two timescales may sometimes be related, as frequent freezing and thawing (e.g., on a diurnal or seasonal timescale) will yield very long doubling times because the cell's metabolism, which is required for doubling to occur, is interrupted.

TABLE 5.2 Examples of Cold Environments (and Their Ages) from Which Viable Microorganisms Have Been Recovered

Ancient Material	Age (years)	Investigator
Glacial ice: Greenland	100,000	Sheridan et al. (2003)
Glacial ice: Antarctica	200,000	Abyzov (1993)
Glacial ice: Guliya, China	750,000	Christner et al. (2003)
Permafrost: Arctic	2,000,000 to 3,000,000	Shi et al. (1997)
Permafrost: Antarctica	8,000,000	Tsapin et al. (1999)

NOTE: Controversy exists concerning some of the reports of survival time for microbes in these different habitats, particularly the earlier reports. Such controversy is slowly dissipating as more microbial studies are conducted on icy environments; the emerging picture is that icy environments provide an excellent repository for maintaining stable DNA and other essential macromolecules. See Willerslev et al. (2004a,b) and Priscu and Christner (2004).

long "period of biological exploration" but rather time periods required for global change. Organisms at these very low temperatures are presumably not increasing in number, but they are capable of producing enough metabolic energy to maintain the integrity of their DNA and essential proteins (Christner, 2002; Napolitano and Shain, 2004) over long periods (see Chapter 8, Box 8.1). Table 5.2 summarizes data for microbial survival in certain cold environments on Earth. Microbes have been show to remain viable for several million years in permafrost environments, and arguments have been made that icy environments provide an excellent repository for maintaining stable DNA (Willerslev et al., 2004a,b). These viability estimates may have implications for potential life in the martian ice caps, which are thought to be ~100 million years old (Fisher et al., 2002).

Permanent Antarctic Lake Ice

The permanently ice-covered lakes of the McMurdo Dry Valleys may be analogs of ancient martian systems (McKay and Stoker, 1989; McKay et al., 2005). Besides an abundance of microbial life in the liquid water columns (e.g., Priscu et al., 1999a), the ice covers themselves have been shown to harbor a microbial consortium of cyanobacteria and bacteria (Priscu et al., 1998; Paerl and Priscu, 1998). These microbes enter the ice on windblown sediments, where they melt into the ice and create liquid water inclusions at a depth approximately 2 m beneath the surface (Priscu et al., 1998) (see Figure 5.1). DNA hybridization studies have shown that the organisms within the ice are indeed seeded from the terrestrial environment rather than from the liquid water column on which the ice floats (Gordon et al., 2000). Once the microbes are bound within the ice cover, they can grow with doubling times averaging 2.5 years, depending on the temporal availability of liquid water within the ice cover (Fritsen and Priscu, 1998). Cyanobacteria supply the consortium with reduced organic carbon and nitrogen via photosynthesis and N_2 fixation, and the heterotrophic bacteria recycle carbon and nitrogen back to the cyanobacteria (Priscu et al., 2005). The close spatial and temporal coupling of metabolites within the microbial consortium is essential for the microbes to survive and replicate in what has been characterized as "the edge of life" (Paerl and Priscu, 1998).

Cryoconite Holes

Cryoconite holes form when dark windblown particulate matter accumulates on the surface of a glacier. Solar radiation warms these particles, causing them to melt into the ice, producing a cylindrical basin of liquid water (see Figure 5.2). The cryoconites may (1) remain liquid on warm sunny days, (2) form an ice cover when air temperature drops and solar radiation persists, or (3) freeze completely during winter when solar radiation is low or absent. As with permanent lake ice assemblages, photosynthesis and N_2 fixation by algae and cyanobacteria supply sufficient reduced carbon and nutrients to support the development of complex microbial ecosystems (Christner et

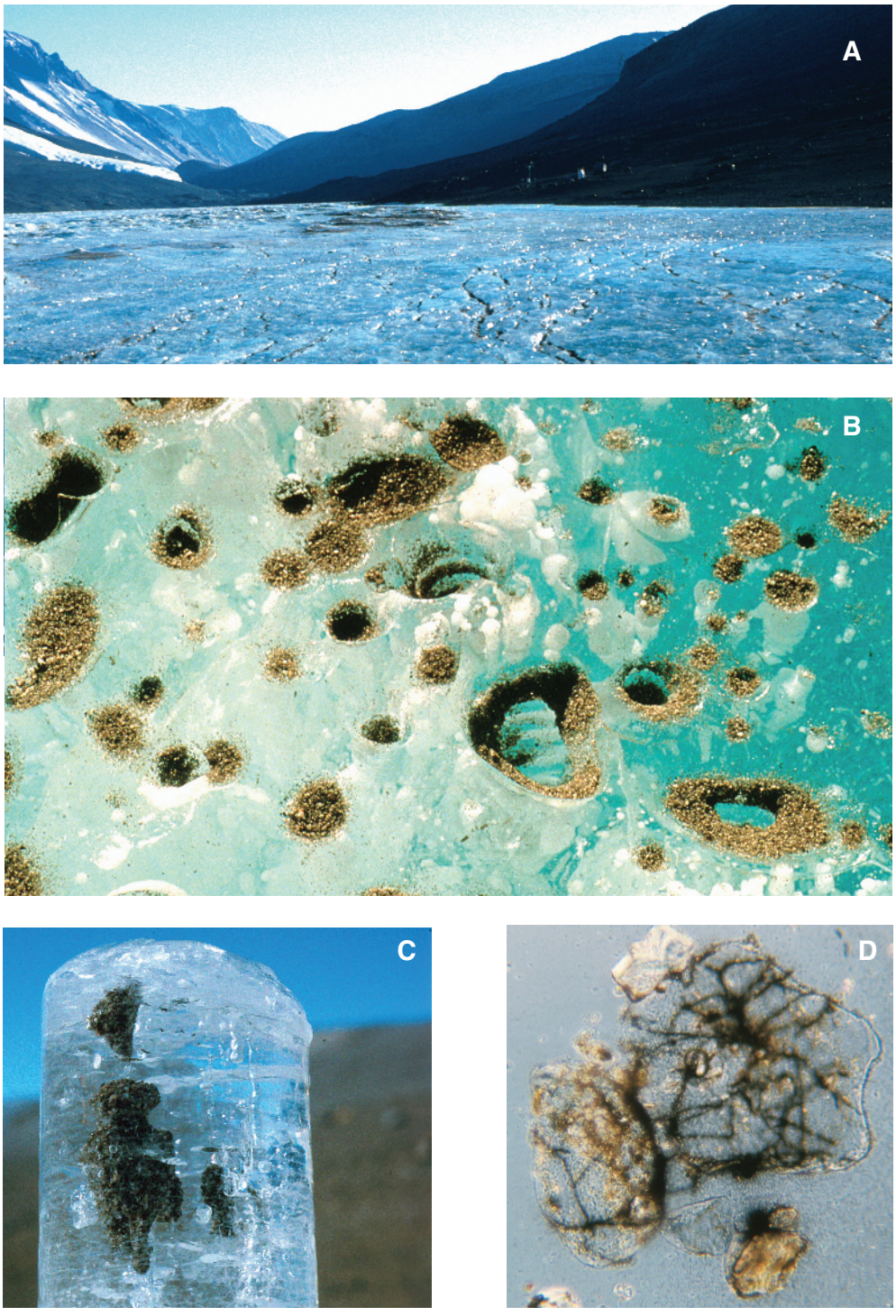

FIGURE 5.1 Sequence of images showing (A) the surface of the Lake Bonney ice cover, (B) a close-up of the surface of the ice cover showing aeolian sediment accumulation, (C) an ice core from 2 m beneath the surface of the ice cover showing sediment accumulation, and (D) microscopic view (1000×) of sediment grains and associated filamentous cyanobacteria. Images courtesy of H. Paerl, University of North Carolina at Chapel Hill, Institute of Marine Sciences.

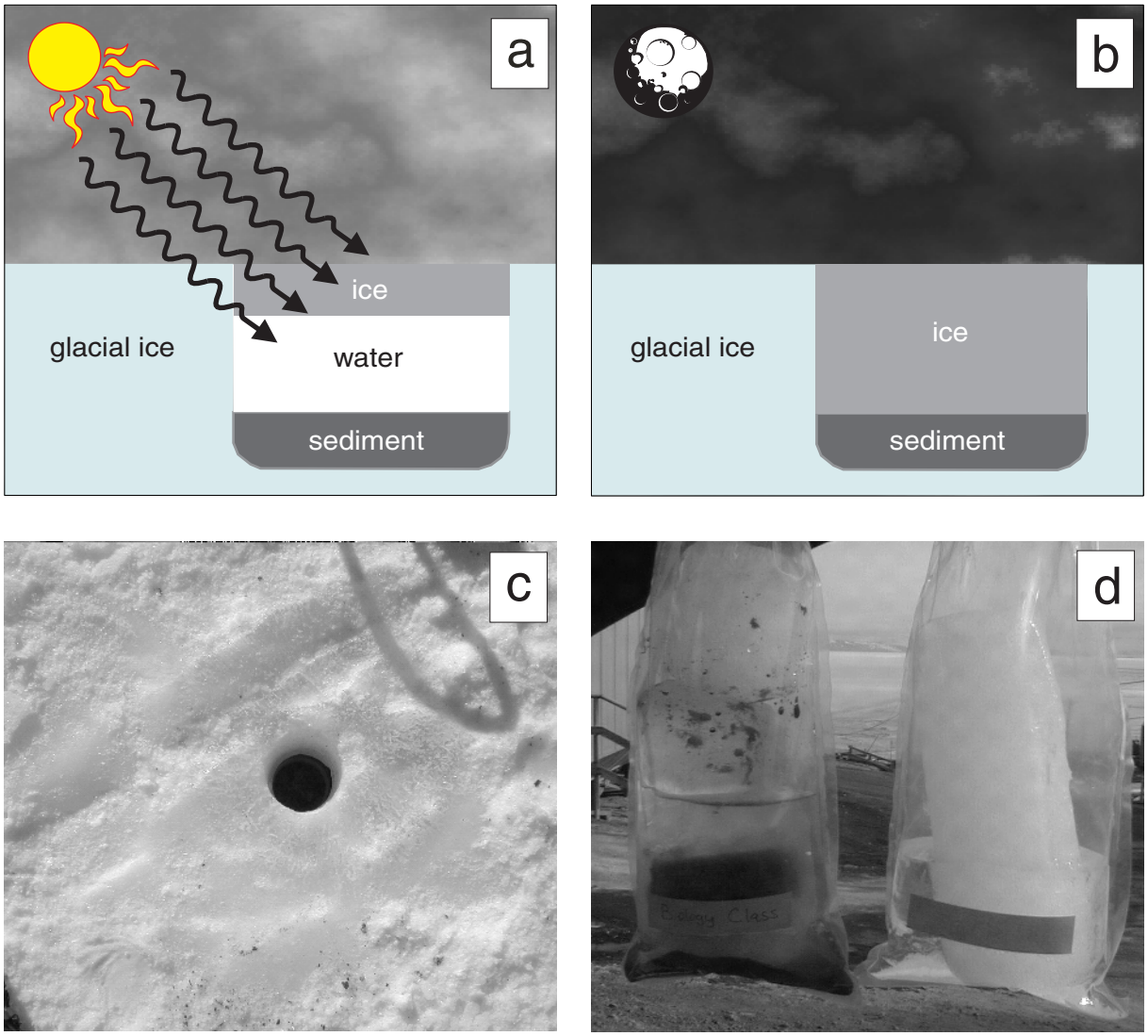

FIGURE 5.2 The cryoconite hole environment in the McMurdo Dry Valleys. (a) In summer, sediment collects on glacial surfaces, and exposure to solar irradiation produces melt pools within the ice, which may subsequently freeze on the surface, and (b) completely freeze during the winter. The 10-cm-diameter cryoconite hole shown in (c) was located on the Canada Glacier and was completely frozen when sampled in January 2001. (d) A comparison of cores retrieved from the cryoconite hole (left) with a core from the adjacent glacial ice. Note the dense layer of sediment and organic material present within the bottom 5 cm of the cryoconite hole core. The diameters of cryoconites on this glacier range from several centimeters to 1 m. SOURCE: Priscu and Christner (2004), ASM Press, Washington, D.C.

al., 2003). The organic matter produced in the cryoconites can seed the surrounding environment during glacial ablation cycles. Cryoconite hole ecosystems occur globally, being found in Arctic (Mueller et al., 2001), Antarctic (Wharton et al., 1981; Tranter et al., 2004), and in temperate alpine glaciers (Takeuchi et al., 2000). Although dominated by microorganisms, cryoconite holes are one of the few Antarctic terrestrial environments inhabitable by metazoan life. A phylogenetic survey of a cryoconite found in the McMurdo Dry Valleys of Antarctica showed that these ecosystems are inhabited by species quite similar to those in adjacent microbial mat and lake ice

assemblages in this polar desert environment (Christner et al., 2003). The high winds in the Antarctic desert ecosystems disperse organic matter throughout the ecosystem and also provide the biological seed for cryoconite holes and lake habitats. As with the permanent lake ice in the polar deserts of Antarctica, cryoconites serve as biological refuges in an environment that would appear to be inhospitable for life.

Glacial Ice

Earth's expansive polar ice caps cover ~10 percent of the terrestrial surface with ice and contain ~70 percent of the freshwater on the planet (Patterson, 1994). Archived chronologically within glacial ice are samples of the atmospheric constituents from different times in the past, including biological material such as insects, plant fragments, fungal spores, viruses, and bacteria (Willerslev et al., 1999; Rogers et al., 2005). Studies indicate that ice core samples from nonpolar, high-altitude glaciers contain a greater number and variety of culturable bacterial species than do polar ices (Priscu and Christner, 2004). The difference between polar and nonpolar glaciers results from increased microbial deposition on glaciers contiguous to environments that supply airborne sediments, which serve to transport and protect attached microorganisms. Aerosolized microorganisms can travel large distances on atmospheric currents, often in a viable but dormant state. Remarkably, some atmospheric conditions actually provide a medium for growth, and microbial metabolism has been detected in fog particles (Fuzzi et al., 1997) and super-cooled clouds (Sattler et al., 2001). For an airborne microorganism deposited in glacial ice to retain viability, the stress associated with desiccation, solar irradiation, freezing, an extended period of no growth, and subsequent thawing must not result in a lethal level of unrepairable cellular damage. It is therefore not surprising that many species isolated from glacial ice form spores, structures known to confer resistance to environmental abuses. Many also have thick cell walls or polysaccharide capsules, and they resist repeated cycles of freezing and thawing. Regardless of the ice core's geographic source, related but not identical species are frequently recovered. Members of the bacterial genera *Sphingomonas, Acinetobacter*, and *Arthrobacter* are commonly isolated from glacial samples (Christner et al., 2000, 2001, 2003; Priscu and Christner, 2004) (see Figure 5.3), and they are also the most frequently isolated genera in enrichment surveys of terrestrial subsurface environments (Balkwill et al., 1997). Accordingly, these genera would appear to contain species that can survive for extended times under low-nutrient, nongrowth conditions, and similar survival strategies may be in effect in deep ice and subsurface situations.

Subglacial Lakes

Much attention is currently focused on the possibility that the subglacial environments of Antarctica may harbor microbial ecosystems under thousands of meters of ice, isolated from the atmosphere for as long as the continent has been glaciated (20 million to 30 million years; Naish et al., 2001). The present lake inventory reveals more than 140 Antarctic subglacial lakes (Priscu et al., 2003). Curiosity about the nature of these environments has intensified as a result of the discovery that Lake Vostok, the largest subglacial lake yet known (Studinger et al., 2004), may harbor microbial life (Karl et al., 1999; Priscu et al., 1999b; Bulat et al., 2004). Molecular profiling (16S rDNA) of water from Lake Vostok that has frozen to the bottom of the ice sheet showed close agreement with present-day surface microbiota (Priscu et al., 1999b; Christner et al., 2001). Phylotypes have mapped closely to extant members of the *Alpha-* and *Betaproteobacteria* and to the *Actinomycetes*, the latter of which was also isolated in Vostok glacial ice (Abyzov et al., 1998). These data imply that microbes within Lake Vostok do not represent an evolutionarily distinct subglacial biota (Siegert et al., 2001, 2003). The timescale of isolation within Lake Vostok (~20 million to 25 million years) is not long in terms of microbial evolution compared with its ~3.5 billion-year history on Earth; studies of species divergence of other microorganisms have shown that species-level divergence may take ~100 million years (Lawrence and Ochman, 1998). However, other mechanisms of genetic change (e.g., recombination and mutator genes) could allow more rapid alteration of organism phenotype, allowing for adaptation to conditions within Lake Vostok (Page and Holmes, 1998). Priscu and Christner (2004) used estimates of microbial biomass in subglacial lakes and the polar ice sheets to show that these poorly described systems may contain a previously unrecognized pool of organic carbon similar in magnitude to all of Earth's surface freshwater ecosystems combined.

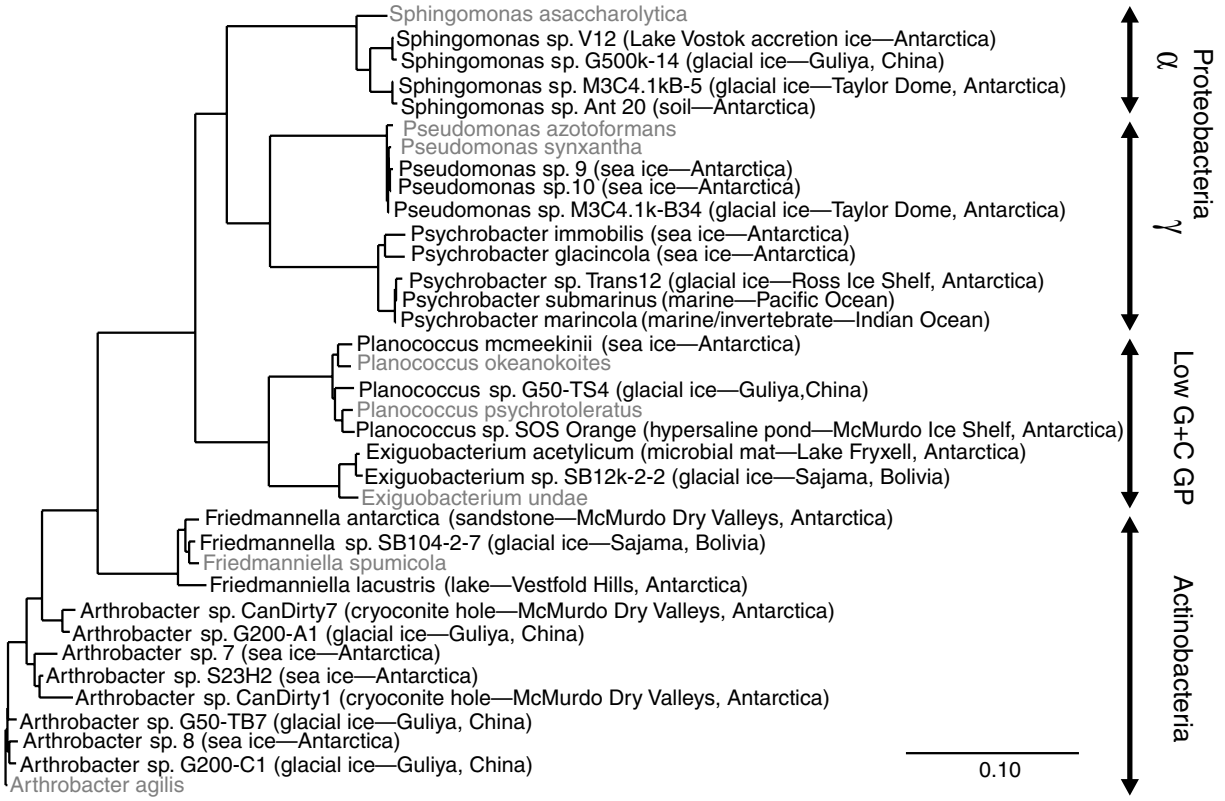

FIGURE 5.3 Phylogenetic analysis of bacteria obtained in microbiological surveys of permanently cold and frozen environments. Isolates from cold habitats are shown in bold, followed by the source environment and geographical location. The 16S rDNA sequences corresponding to nucleotides 27-1492 of the *E. coli* 16S rDNA were aligned based on secondary structure and used to construct this neighbor-joining tree. The scale bar represents 0.1 fixed substitutions per nucleotide position. SOURCE: Priscu and Christner (2004), ASM Press, Washington, D.C.

Permafrost

Permafrost is defined as ground that has maintained a temperature lower than 0°C continuously for more than 2 consecutive years. Some permafrost ecosystems are thousands or millions of years old and can serve as "ecological time capsules," since they may contain micro- and macro-organisms trapped at the time of freezing (Gilichinsky et al., 1992; Tsapin et al., 1999). For example, conspicuous flora and fauna such as bison, horses, and mammoths have been detected by their preserved DNA signatures (Willerslev et al., 2003).

Many of the microorganisms embedded in permafrost soils may remain viable and amenable for cultivation even after hundreds of thousands of years (Vishnivetskaya et al., 2000; see also Table 5.2). This allows for the study of microbial strains that may have been sequestered from ecological interactions with contemporaneous biota for relatively long periods of time. There are multiple physiological adaptations unique to permafrost microbes that can be studied in isolated strains, for example, extremely slow grow rates at –10°C (Bakermans et al., 2003; Rivkina et al., 2000). In addition, other in situ measurements of metabolic activity (e.g., glucose incorporation, methanotrophy) and amino acid chirality support the view that permafrost microbial assemblages are viable and metabolically active in situ.

The combination of low temperatures and relatively high salt content extruded from permafrost soil provides a unique terrestrial hypersaline environment in the form of brine lenses or cryopegs that can reach temperatures of

–15°C and still display metabolic activity in the form of radio-labeled-glucose uptake (Gilichinsky et al., 2003). All these diverse permafrost ecosystems provide terrestrial models for understanding slow rates of microbial growth, low-temperature adaptations, dormancy, and cryoprotection mechanisms that can serve as analogs to other extraterrestrial systems.

Survival in Cold Environments

A phylogenetic comparison of species inhabiting icy environments shows similar phylogenetic 16S rDNA profiles (and therefore the presence of similar kinds of microbes) from Antarctica and other permanently cold nonpolar locales (see Figure 5.3) (Priscu and Christner, 2004). The psychrophilic and psychrotrophic isolates shown in Figure 5.3 originate from locations ranging from aquatic and marine ecosystems to terrestrial soils and glacial ice, with little in common between these environments except that all are permanently cold or frozen. These data argue that clades[13] in these bacterial genera evolved under cold conditions and may possess similar strategies to survive freezing and remain active at low temperature. A recent thermodynamic analysis suggested that there is no minimum temperature for metabolism, and the rate at –40°C in ice corresponds to ~10 turnovers of cellular carbon per billion years (Price and Sowers, 2004). Price and Sowers (2004) concluded that microbes in ice and permafrost have metabolic rates similar to those of microbes in water, soil, and sediment at the same temperature. This contention supports the view that, far below the freezing point, liquid water inside ice and permafrost is available for metabolism. As discussed in Chapter 4, such conditions may exist in the martian subsurface. Organisms living in such environments must be able to withstand both the cold conditions and the high salts that often concentrate in the grain boundaries within these systems (Price, 2000). Owing to the difficulty encountered when culturing true psychrophiles and their slow growth rates in the laboratory, relatively little is known of the adaptive mechanisms possessed by this group of microorganisms.

Hot Environments

Many thermophilic organisms (such as the *Aquificales*, *Thermotogales*, *Sulfolobales,* and *Archaeoglobales*) are representatives of the deep branches of the Archaea and Bacteria, and they are known only from thermal environments. Some of these groups have mesophilic and even psychrotrophic relatives from the gram-positive bacteria[14] (including the spore-forming *Bacillus*), *Verrucomicrobiales*, and from the methanogens. Heterotrophic organisms such as *Geobacillus stearothemophilus* and representatives of the *Thermotogales* are often present in hot environments, but many of these ecosystems are oligotrophic and are dominated by autotrophs that use H_2, S, NH_4^+, and other substrates as electron donors. These ecosystems are often anoxic or contain very low concentrations of oxygen, increasing the importance of alternative terminal electron acceptors such as NO_3^-, Fe (III), and S (SO_4^{-2}, S, and S_2O_4). Furthermore, some organisms are even capapble of inorganic fermentation (disproportionation) using sulfur or sulfite (D'Hondt et al., 2002).

Terrestrial Thermal Environments

Hot springs such as those found in Yellowstone National Park and in Iceland, New Zealand, and Japan have provided scientists with habitats on Earth to study the upper temperature limits of life (Figure 5.4). Brock and other researchers were among the first to describe the physiologies of organisms that could survive and multiply within environments approaching or exceeding boiling temperature (Brock et al., 1972; Brock, 1978). The thermophile *Thermus aquaticus* has been found in thermal habitats throughout the world. Its temperature range is about 50 to 80°C, and its optimum temperature for growth is around 70°C. Its temperature range overlaps that of the photo-

[13]A clade is a group composed of all the species descended from a single common ancestor, that is, a monophyletic group.

[14]This classification relies on differences in the cell wall components that can be differentiated by stains that detect peptidoglycan in gram-positive cells and lipopolysacharide in gram-negative cells.

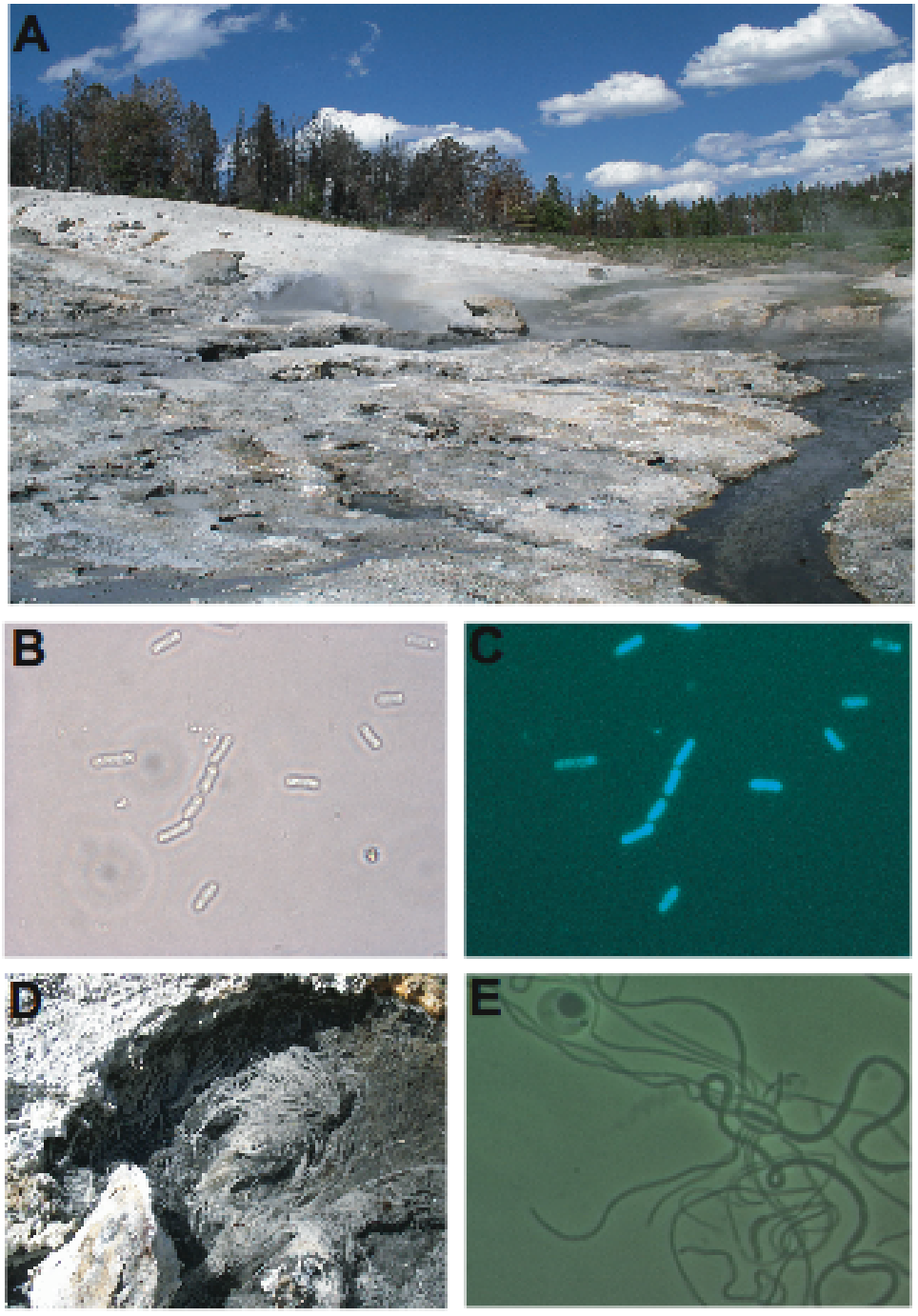

FIGURE 5.4 (A) Coffee Pots, a near-neutral thermal spring in Yellowstone National Park. Resident microbes include phototrophs, chemoautotrophs, and heterotrophs. (B) and (C) are images of the same field of view, except that the cells in Panel C were viewed using epifluorescent microscopy, causing them to autofluoresce and indicating that they contain photosynthetic pigments. (D) *Aquificales* filaments are macroscopic and resemble tufts of hair attached to thermal spring substrate. (E) Although *Thermus sp.* have not been detected in this spring, they are found throughout Yellowstone National Park and terrestrial thermal springs worldwide. All micrographs are 1000×.

synthetic bacteria, so that in many hot springs it lives in association with cyanobacteria, ultimately obtaining its energy for growth from the photosynthesis of these organisms. However, it may also be found in lower-diversity environments where temperatures are too high for photosynthesis.

Numerous studies have now described the diversity and productivity of the microbial biota found in geothermal ecosystems. Ecological studies of terrestrial hot-spring microbial communities have reshaped views of microbial biodiversity and of the composition, structure, and function of microbial communities (e.g., Ward et al., 1998). Terrestrial hot-spring microorganisms were among the first extremophiles to be surveyed using molecular technology (Stahl et al., 1985; Ward et al., 1990) and thus were among the first in which the impressive diversity of uncultivated microbial populations in nature was revealed. This has been a typical finding in 16S rRNA gene surveys of microbial diversity in numerous habitats (e.g., Bintrim et al., 1997; Borneman and Triplett, 1997). For example, 16S rRNA studies of hyperthermal hot-spring habitats have led to the discovery of novel uncultivated bacteria (Reysenbach et al., 1994; Pace, 1997) and archaea (Pace, 1997; Barns et al., 1996) that are particularly interesting because they branch near the root of 16S rRNA-derived phylogenetic trees. Because such microorganisms may help researchers to determine characteristics of the most ancestral cells, it is also noteworthy that many of them have been brought into culture (Huber et al., 1995, 1998).

Deep-Sea Hydrothermal Environments

The deep sea was traditionally considered a virtual biological desert. Because phytoplankton, the ocean's primary producers, are restricted to the upper water column of the sea, the abyssal zone lying at the bottom of the ocean was assumed to be incapable of supporting a significant biological community. However, exploration off the coast of Ecuador in 1977 (Corliss et al., 1979) revealed a diverse community of both prokaryotes and eukaryotes thriving within and surrounding deep-sea hydrothermal vents. Well outside of the ocean's sunlit photic zone, chemoautotrophic archaea and bacteria, which obtain their energy from the oxidation of inorganic chemicals and fix carbon dioxide for growth, were shown to be the primary producers in deep-sea vent communities, rather than photosynthetic organisms (Karl et al., 1980). The total potential chemosynthetic production for deep-sea hydrothermal ecosystems is estimated to be about 10^{13} g biomass per year, which represents approximately 0.02 percent of global primary production by photosynthesis in the oceans (McCollom and Shock, 1997).

Deep Subsurface Environments

Early reports of microbes cultivated from Earth's deep subsurface, primarily from gold mines and oil fields, were met with skepticism because of concerns about contamination. However, improved sampling techniques and the fact that the physiological characteristics of the cultures were consistent with the energy sources and thermal conditions from which they were isolated corroborated the authenticity of their origin (Pedersen, 2000). Early hypotheses suggested that life could extend as much as 10 km deep (Gold, 1999), and preliminary data suggest that microbial communities exist at least 3 to 5 km below Earth's surface (D'Hondt et al., 2002; Lehman et al., 2001; Fredrickson and Onstott, 1996; Szewzyk et al., 1994; Whitman et al., 1998). Currently, depth is not believed to be the factor limiting subsurface microbial distribution. Rather, it is increased temperature with depth that may restrict life (Pedersen, 2000). The maximum known temperature for microbial growth in the laboratory is 121°C (Kashefi and Lovley, 2003), which is not reached before 5 to 10 km or more in shield rocks, mountains, and deep sediments. The true maximum temperature for life in situ is still unknown, but it is theoretically 150°C (Stetter, 1998). Although surface soil and sediment microbial biomass decrease with depth, some deep subsurface environments harbor microbial abundances as high as 10^5 to 10^6 cells cm^{-3}; abundances often increase with increased carbon and energy sources. The vast extent of this biological niche, both below continents and the ocean, results in estimates of subsurface biomass that may account for more than 90 percent of global microbial biomass, 60 to 100 percent of plant biomass, or 3 to 5×10^{17} g of carbon (Whitman et al., 1998; Pedersen, 2000). Although these estimates are based on very preliminary data—and spatially this niche is presumably highly variable—it is clear that deep subsurface biomass is significant and potentially quite large.

In the deep subsurface, a hydrogen-driven biosphere is hypothesized in which hydrogen is formed by either the reaction of gases dissolved in basaltic magma, decomposition of methane (CH_4) to C and H at temperatures above 600°C, or radiolysis of water by radioactive isotopes. Under these circumstances, methanogens and acetogens form the basis of the community's food web, producing methane and acetate, respectively. Claims for the discovery of hydrogen-driven communities within Earth's deep subsurface have encouraged the search for subsurface life on other planets (Stevens and McKinley, 1995; Chyba and Hand, 2001; Chapelle et al., 2002).

Survival in Hot Environments

Archaea and bacteria dominate hydrothermal ecosystems because temperatures often exceed the habitable limits of eukaryotes (~62°C) and photosynthetic microorganisms (~73°C) (Brock, 1994). The dominance of archaea and bacteria is related in large part to their structural simplicity and metabolic diversity (eukaryotes can be considered structurally complex and metabolically limited). At first, the microbial diversity in hot environments was thought to be relatively low, owing to what was perceived as an extreme environment, a conclusion based primarily on the limited information provided by culturing methods. However, the application of in situ molecular approaches (e.g., characterization of small-subunit rDNA gene diversity) showed that thermal communities consisted of representatives from many of the bacterial and archaeal divisions and led to the discovery of new phylogenetic groups (Barns et al., 1996; Hugenholtz et al., 1998).

The recently discovered hyperthermophile phylum Nanoarchaeota appears to represent yet another deeply branched, independent lineage in the archaean domain. It is unrelated to any cultivated microbial group or environmental sequence.[15] Representatives of nanoarcheota were first found in thermal samples from Iceland and were co-cultivated with *Ignicoccus*, another hyperthermophilic archaean of larger size (Huber et al., 2002, 2003). Preliminary surveys to detect nanoarchaea have shown that this lineage may be much more diverse and also widely distributed in hydrothermal features where other hyperthermophilic archaea such as *Ignicoccus* exist (Hohn et al., 2002). Recent research further showed that there are at least five lineages clustered by location in thermal sites with temperatures ranging from 70 to 90°C (Chang et al., 2004). Both their genome and cell size are the smallest yet documented, and analyses of their entire genome revealed that many of the genes required for a free-living mode of life are missing, which explains nanoarchaea's dependence on their host for survival (Waters et al., 2003). Comprehensive environmental studies linked with attempts to cultivate these microbes will provide new insights into the minimal number of genes required for microbial life in symbiotic or parasitic interaction with their hosts. Such studies may shed some light on the range of physiologies and the adaptations required to exist at temperatures close to 100°C and in the presence of S, H_2, and CO_2.

Desiccating Environments

The Atacama Desert of northern Chile is a high (with most elevations over 2,500 m) and cold desert, with a temperature range of 0 to 25°C. After Antarctica, the Atacama is considered the world's driest region. Although the Tropic of Capricorn passes through the region, the Atacama lies in the rain shadow of Chile's Coast Range, which removes the moisture from the atmosphere. (Extremophilic microorganisms that are capable of growing under conditions of low water activity (effective water content expressed as mole fraction) are called xerophiles.) The desert appears completely barren. Most areas receive moisture only from an occasional fog or a shower every few decades. Measurable precipitation rarely occurs, and some places in the area have not had precipitation for hundreds of years. This lack of precipitation places the Atacama environment near the threshold that will support life, as even extremophiles are in low numbers in this environment (Navarro-González et al., 2003). Navarro-González et al. (2003) showed that samples from this Mars-like region had trace levels of total organic matter and

[15]PCR experiments that employed universal rRNA primers failed to detect *Nanoarchaeum equitans* in molecular diversity studies (Huber et al., 2002).

extremely low levels of culturable bacteria. Epifluorescence microscope counts also showed relatively low bacterial abundances of 0.7×10^6 cells per gram and 9.6×10^6 cells per gram for the surface and subsurface Atacama samples, respectively (typical densities in garden soils are 3 to 4 orders of magnitude higher) (Glavin et al., 2004). These epifluorescence counts are much higher than total counts of viable, culturable heterotrophic bacteria (10 to 10^4 colony-forming units per gram) previously measured by serial dilution plating (Navarro-González et al., 2003), indicating that soil samples from the Atacama Desert contain mostly nonculturable bacteria that are not detected by dilution plating.

Although bacteria in the Atacama soils are near the detection limit of standard analytical techniques, the results of Glavin et al. (2004) imply that the subsurface sample contains a higher total organic content and a higher bacterial cell concentration than do the surface soils. This gradient may result from the presence of a concentration gradient of oxidants with depth. The presence of highly oxidizing conditions within the soils of the Atacama was corroborated by incubation experiments patterned after the Viking labeled-release experiment, but with separate biological and nonbiological isomers. These experiments showed active decomposition of organic species in these soils by nonbiological processes (Navarro-González et al., 2003). The low levels of organic matter in the Atacama Desert in concert with its oxidizing conditions make it an analog for conditions on Mars that offers a potential testing ground for decontamination and life-detection studies.

PROBABILITY OF GROWTH ON MARS

Can we combine our knowledge of the martian environment with our knowledge of extremophilic microorganisms to estimate the probability of growth (P_g) of Earth organisms on Mars? In Chapter 2 (see also Appendix D), it is noted that past estimates of P_g for particular martian environments have ranged as low as 10^{-10}, a value assigned to subpolar regions of Mars within 6 cm of the surface (NRC, 1978). In light of the findings about the martian environment described in Chapter 4 and the growing knowledge of terrestrial psychrophiles and psychrotrophs described in this chapter, the committee suspects that such extremely low values give a false sense of confidence about the risks of microbial contamination of Mars. However, quantifying this suspicion remains very difficult.

Estimates of P_g must account for the physiological tolerances and relative numbers of each kind of microbe on the spacecraft, with special emphasis on extremophiles that might be capable of survival and growth in a specific extraterrestrial environment. As described in Chapter 2, current NASA planetary protection practices for Mars do not consider physiological and genetic diversity, and bioburden estimates fail to differentiate between different kinds of microbes. As described in this chapter, molecular investigations of microbial population structures reveal that many organisms in natural environments are difficult if not impossible to grow in the laboratory. These discoveries have important implications both for knowledge of microbial diversity and inferences about microbes' ability to survive conditions on Mars, where liquid water might occur.

Estimates of biodiversity based on molecular techniques imply that under controlled nutrient concentrations, salinity, pH, temperature, and so on, microbiologists have successfully cultured fewer than 10 percent and possibly as little as 0.1 percent of the different kinds of organisms from natural settings (Ward et al., 1990). The likelihood of growth in a given environment on Mars may be orders of magnitude lower because experiments involving the accidental delivery of Earth organisms to Mars do not permit the controlled manipulation of environmental conditions that is common during successful attempts to culture microbes on Earth. The probabilities of meeting all conditions necessary to support the growth of an introduced microorganism to the martian surface or subsurface imply that estimates for the probability of growth for a particular kind of microorganism on Mars (P_g^i, where the superscript i denotes the i^{th} kind of microorganism) may range from 10^{-3} to much lower values. For these reasons, it seems unlikely that P_g for an arbitrary organism would exceed 10^{-3}, and in fact it could be much lower, given the potential hostility of many martian environments.

Given sufficient resources, molecular techniques could be used to assess microbial diversity Δ on the spacecraft (see Chapter 6). With information about the kinds of organisms present, one could write Equation 2.1 more rigorously, taking into account the varieties of microorganisms i present on the spacecraft:

$$P_c = \sum_{i=1}^{\Delta} N_0^i R^i P_S^i P_I^i P_R^i P_g^i \tag{5.1}$$

where the variables have the same meaning as in Equation 2.1, except indexed by i to a particular type of microorganism. As before, Equation 5.1 assumes that P_c is small compared with unity. Historically, in the absence of modern DNA phylogenetic methods, there was nearly no knowledge of the varieties i of microorganisms on spacecraft. Instead, because it was believed that spores would be the most likely organisms to survive first interplanetary transport and then the martian surface, Equation 5.1 was collapsed down to culturing sporulating bacteria only, estimating P_c on this basis, provided that P_g for these organisms could be estimated (see Chapter 2).[16]

To employ P_g to determine bioburden reduction requirements for Mars missions would require knowing enough about Mars and Earth microbes to assign quantitative values to the terms in Equation 5.1. Clearly, research over the past 10 years has broadened researchers' views on the diversity of extreme habitats in which microorganisms can survive and reproduce on Earth. This advancement is due in part to methodological breakthroughs (primarily in genomic identification and characterization) and a change in research focus by national funding agencies. Knowledge of the capability of Earth microorganisms to survive and propagate on Mars, however, remains quite limited.

Research on extremophiles is still in its infancy, and there is much more to be learned about the physiology and ecology of these organisms. Rapidly changing views on microbial survival and propagation in Earth environments, in concert with considerable uncertainties in knowledge about the martian situation (see Chapter 4), make P_g a scientifically nontractable variable today. Scientists would be hard pressed to accurately compute P_g for microorganisms on Earth; to compute P_g for Earth microorganisms in still poorly understood martian environments currently remains too great a challenge. However, if we were to gain detailed knowledge of the microbial populations on spacecraft (using the techniques described in Chapter 6), and further knowledge of the martian surface and subsurface, most terms in Equation 5.1 might be confidently set to zero, because $P^i{}_S$ and $P^i{}_g$ may well be extremely close to zero for nearly all kinds i of microorganisms on spacecraft. Were this to prove to be the case, spacecraft bioburden reduction would then have to be applied only to those particular kinds of microorganisms for which $P^i{}_S$ and $P^i{}_g$ were suspected or known to be appreciable. This might allow an approach to bioburden reduction very different from those employed today. If, for example, future research were to demonstrate that psychrophilic and psychrotrophic extremophiles were universally inactivated (killed) at temperatures low compared with those used in the Viking mission protocols (see Chapter 2), spacecraft might be able to be heat sterilized at much lower temperatures, with fewer consequences for spacecraft material and components.

SUMMARY

Growth and division are the factors required for successful survival in any environment. The fact that microorganisms not only survive but also actually grow in some of the harshest environments on Earth has stimulated scientific curiosity about the evolution and physiology of the organisms that remain viable in this diverse range of temperatures, pH levels, pressures, and salt concentrations. It has also produced a plethora of new research on what have become known as extremophiles. The use of molecular methods to unravel the phylogenies of microorganisms within extreme environments has shown that most of these environments are populated by bacteria, archaea, and unicellar eukarya, which have genetically encoded metabolic plasticity to cope with many divergent environments on Earth. The genomic era has now revealed how extremophiles differ from ordinary microorganisms,

[16]The approach taken for Europa in the NRC report *Preventing the Forward Contamination of Europa* chose $\Delta = 4$ in Equation 5.1, allowing in principle the consideration of four broad classes of microorganisms that might be present on the spacecraft: common microorganisms, spores, spores that are especially radiation resistant, and highly radiation-resistant nonspore microorganisms (NRC, 2000, p. 29).

leading to new concepts of microbial evolution and providing impetus to apply genomic methods to research on extremophiles.

The use of molecular methods has revealed that microbial diversity far exceeds what scientists have been able to culture in the laboratory. Genomic tools have made it possible to probe many environments on Earth that were previously thought to be devoid of life. We now know that life occurs in almost every environment on our planet that contains liquid water. Despite that newfound knowledge, the limits to life and its microbial diversity on Earth have not yet been completely defined. Although significant uncertainties remain about the physical and chemical conditions on Mars, it does appear that some subsurface martian conditions are within the known limits of Earth life (see Chapter 4).

The cold temperatures on Mars and the paucity of persistent liquid water would provide selective pressure for the persistence of psychrophilic and psychrotrophic organisms, much as seen within the icy systems on Earth. Earth psychrophiles could survive long-term in the martian environment, but they would not grow rapidly. Data from a variety of cold environments on Earth (see Table 5.2) show that microorganisms can remain immured in ice for many millions of years and remain viable. Willerslev et al. (2004a) concluded that the life-essential nucleic acids DNA and RNA should in theory survive for millions of years in ice and permafrost and remain intact and extractable. At the lowest temperatures of any geologic setting, glacial ice and permafrost are likely to contain the oldest endogenous nucleic acids on Earth.

An understanding of conditions on Mars and the requirements that Earth organisms face for growth in different environments is at the heart of planetary protection and the implementation of planetary protection policy. Chapter 6 describes modern molecular techniques that could be applied to understand the diversity of microbes on spacecraft and methods for eliminating them.

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6

Advances in Technologies for Life Detection and Bioburden Reduction

At present, the most stringent bioburden reduction requirement is Viking post-sterilization (see Table 2.1), which imposes costs in resources and time. At the same time, new information from studies of microbial diversity suggests that NASA's standard microbial monitoring procedures may underestimate the number of organisms that could compromise planetary protection efforts. This chapter examines new technologies that can aid in the detection and identification of living microorganisms and that may play a role in planetary protection, particularly in the analysis and reduction of bioburden[1] in spacecraft assembly areas and on the spacecraft.

Ultimately, the use of advanced detection methods to identify the potential bioburden may lead to reduction techniques that are targeted at groups of organisms of specific concern for planetary protection. NASA is supporting research on some of these technologies (e.g., Dickinson et al., 2004a,b; Venkateswaran et al., 2001, 2003).[2]

EXAMPLES OF METHODS FOR ASSESSING TOTAL VIABLE CELL COUNT

Previously, a major obstacle to detecting and identifying microorganisms has been the limited capabilities of the available methodologies. Such microorganisms are of microscopic size and have relatively few morphological differences, and so detecting them and distinguishing individual taxa usually required the isolation and growth of microorganisms in the laboratory as pure cultures for metabolic and physiological investigations. However, as described in Chapter 5, most microorganisms are not amenable to laboratory culturing, and culturing is not an effective method for assessing the types of microorganisms in situ. Fewer than 1 percent of organisms in the environment are directly culturable using current techniques (Amman et al., 1995), and these organisms often represent minor members of natural populations in terms of both their numbers and their role in ecosystem processes (Ward et al., 1998). It is unlikely that the current NASA swab and culture approach is more successful at growing all spore formers present on spacecraft. Organisms that can be cultured in the laboratory are those that are most compatible with laboratory media, as opposed to organisms that have optimally adapted to a particular environment. The development of high-throughput culture conditions that mimic environmental conditions has reduced the bias of culturing microorganisms (Connon and Giovanonni, 2002; Kaeberlein et al., 2002), but this

[1]Bioburden, or total viable cell count, refers to the number of microorganisms on or in a contaminated object.

[2]See also Baker (2001), Baker and Rummel (2005), and Kminek and Rummel (2005).

laborious technology remains ineffective in assessing the total bioburden of spacecraft, since some microorganisms may still elude cultivation.

The current NASA approach (described in Chapter 2) of using the cultivation of spore-forming bacteria does not provide an accurate estimate of total bioburden. Moreover, it is possible that some of the organisms of potentially greatest concern for the forward contamination of Mars, such as psychrophiles (see Chapter 5), may not be well correlated with the counts of spore-forming bacteria on spacecraft. New rapid and less expensive methods that provide more accurate estimates of total viable bioburden, and that do not require growth of microorganisms in the laboratory, are described below.

Cytological Methods

Epifluorescent Microscopy

The use of epifluorescent microscopy[3] and flow cytometry[4] to directly count cells labeled with high-quantum-yield fluorescent nucleic acids stains such as acridine orange, DAPI, and Sybr-Gold has increased estimates of microbial biomass in nature several-fold and has revised understanding of the importance of microorganisms in the natural environment. However, the detection of nucleic acids is not an indicator of viability. Fluorescent stains must be used in concert with other assays that enable the detection of essential metabolites or enzymes, if accurate estimates of total viable biomass are to be obtained.

Detection of Membrane Integrity

Membrane integrity is an indication of cell viability that can be detected using commercial fluorescence stains such as the BacLight Live/Dead kit.[5] This kit uses two nucleic acid stains, SYTO 9 and propidium iodide, which differ in their ability to penetrate healthy cells. SYTO 9 can penetrate both live and dead cells, whereas propidium iodide can only penetrate cells with damaged membranes (dead or damaged cells). When those stains are used together, live cells fluoresce green and dead or damaged cells are red when viewed with an epifluorescent microscopic. Epifluorescent microscopy techniques will require either witness coupons (replicate coupons made of the same material as the spacecraft that can follow the spacecraft through the entire assembly and cleaning process and can be mounted under a microscope for viewing) or microscopic procedures in the clean-room and launch facilities to examine surfaces directly.[6] Irrespective of how cells are stained, the sensitivity of all light microscopy techniques is constrained by the volume of material that can be viewed in a field. At a magnification of 1000×, the fluid volume represented in a viewing field will be ~0.05 μl. There is a 50 percent probability of finding a single cell in a field if the concentration of cells in the original sample is 2×10^3 cells per milliliter.

Flow Cytometry and Cell Sorting

Flow cytometers equipped with a fluorescently activated cell sorter (FACS) analyze particles in a laminar flow by their light-scattering and fluorescent properties.[7] Flow cytometers are used to enumerate and characterize

[3]Epifluorescent microscopy relies on the excitation of susceptible molecules in a sample with short-wavelength, high-energy light and observation of the emitted lower-energy light (fluorescence). Susceptible molecules include autofluorescent compounds such as chlorophyll and F420 (an enzyme cofactor found in methanogens) or fluorophores that bind with specific cell structures. Epifluorescent microscopy enables researchers to observe and detect cellular components that were not visible with conventional contrast microscopy and is sensitive enough to detect a single molecule.

[4]Flow cytometry is the analysis of cells that have been labeled with fluorophores as they pass in a narrow stream through a laser beam.

[5]BacLight is just one of many commercially available kits that may be used for detecting viable biomass.

[6]The suggested technique would be performed on witness coupons and would leave no residue. Should microscopy techniques be developed that could be used to examine the spacecraft directly, any residues would presumably be cleaned before launch.

[7]This technology was originally developed to analyze cell suspensions combined with fluorescently labeled antibodies by conjugated

selected microbial species in environmental samples by applying fluorescent labeled DNA or rRNA probes (see "Examples of Methods Relevant to Estimating Biodiversity" below; see also Amann et al., 1995) followed by selective sorting of labeled cells to further study their morphology and other characteristics.

Biomarker Methods

By focusing on organic and inorganic biomarkers, it is possible to avoid microscopy altogether. Such methods are highly sensitive and employ measurements of selected metabolic products as a proxy for biomass. These procedures are rapid and well suited for repeated use throughout the spacecraft assembly and launch process.

Limulus Amebocyte Lysate Assay

Most bacteria are either gram positive or gram negative. This classification relies on differences in the cell wall components that can be differentiated by stains that detect peptidoglycan in gram-positive cells and lipopolysacharide in gram-negative cells. Cell wall components can also serve as targets for certain biomarkers. For example**,** assays based on the limulus amebocyte lysate (LAL) from the horseshoe crab *Limulus polyphpemus* take advantage of a sensitive enzyme cascade that is triggered by the microbial cell wall components lipopolysaccharide and beta glucan.[8]

The LAL assay can detect 10^{-13} grams of lipopolysaccharide from *Escherichia coli*, which corresponds to one cell. However, the ability to detect a single cell is constrained by the upper limits of the sample size. Typically, each assay can test only 0.1 ml. To be confident at the 98 percent level of detecting a single organism in this small volume, the lower limit of cell concentration would have to exceed 40 cells per milliliter in the sample.[9] The assay detects many gram-negative bacteria and fungi. Signals from gram-positive organisms are likely to represent contamination from soils, which can contain large amounts of gram-positive microbes. The technology is not useful for detection of archaean or eukaryan microorganisms and therefore is not effective in detecting total bioburden. A portable instrument that can produce results in near-real time is currently under development. The LAL assay provides no information about the diversity or viability of microorganisms in a sample, but the combination of speed and sensitivity renders it a potential tool for determining selective bioburdens on spacecraft surfaces. This technique may provide a good real-time indication of gram-negative bacterial life. In combination with molecular phylogenetic analyses (see below) LAL has the potential to detect and provide quantitative estimates of target organisms.

ATP Bioluminescence Assay

Unlike the LAL assay, the adenosine triphosphate (ATP) bioluminescence assay provides a very sensitive indicator of live cells (i.e., cells containing functional ATP). Assays for ATP are able to measure quantitatively the presence of microorganisms in an environmental sample within minutes rather than days. ATP is a stable molecule found in relatively high concentrations in metabolically active cells and it is the primary energy currency in all known living organisms. Commercial ATP-bioluminescence test kits measure the quantum output that occurs when ATP drives the luciferin-luciferase reaction in fireflies. The quantum output from the luciferin-luciferase

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chromophores such as phycoerythrin and green fluorescent proteins. The fluorescence of the particles is then detected in several channels corresponding to the emission peak of the fluorophore and characterized and sorted according to this property.

[8]In this reaction, the cascade produces an activated protease that can be measured using a synthetic peptide substrate coupled to the fluorogen m-coumarin. Upon cleavage, the fluorogen is released and its fluorescence is no longer quenched by the synthetic peptide. The amount of the released fluorogen is proportional to the number of cells in the sample, and it can be measured spectrophotometrically within an hour of taking a sample.

[9]When sampling a cell population that has a concentration of one cell per unit volume, the chances of including a single cell in that unit volume are Poisson distributed. The sampling of a single unit volume would have a 50 percent chance of containing a single cell, whereas a 98 percent probability would correspond to a fourfold sampling of the unit volume.

reaction is directly proportional to the amount of ATP within the cells, which can then be equated to viable cell concentration. Tests are available that can detect approximately 1000 cells. These kits are able to differentiate extracellular ATP from intracellular ATP, which in effect can provide a means to differentiate between live and dead cells. As with the LAL assay, ATP-bioluminescence assays are both rapid and adaptable to small instrument designs. Neither of these assays provides information on the diversity of microorganisms, but both are powerful tools for determining the total bioburden on selected spacecraft components.

A promising advance in detection of ATP is the use of a fusion protein containing ADK plus PolyP kinase (PPK)[10] to amplify the amount of ATP in the sample. Standard bioluminescence can then measure the levels of ATP. Through this amplification mechanism, it is possible to detect ATP levels that correspond to a single cell. For planetary protection purposes, this would offer a rapid and sensitive method to detect a single cell, although the amplification assay is not linear in its correlation between luminescence and initial ATP concentrations. There is also a concomitant increase in the background noise relative to standard bioluminescence assays. This assay is still under development.

Both the LAL and ATP assays are examples of emerging technologies for rapid determinations of numbers of microbes without requiring their cultivation in the laboratory or observation with microscopy. Based on current technology, the limits of life detection can reach about 100 cells/ml,[11] and future advances in LAL and ATP technology and sample handling may one day allow for the detection of one cell per milliliter. The application of these techniques and others to planetary protection will require the development of a standard certification process that can calibrate the relationship between biomarker levels and bioburden as measured in terms of numbers and kinds of organisms.

EXAMPLES OF METHODS FOR ESTIMATING BIODIVERSITY

Measurement of the number of microbial cells on a spacecraft provides a first estimate of total bioburden, but additional information is needed if planetary protection requirements are to be sensitive to organism type. For example, the ATP and membrane integrity assays will not detect spores, owing to their dormant state. More important, these estimates of biomass do not provide information about the kinds of organisms present on the spacecraft—important information to evaluate the potential for organisms to survive or propagate on Mars. Studies based on metagenomic analysis (sequence-based or function-based screening and analysis of genomes from a mixed assemblage of microorganisms) will one day provide a means to correlate physiological properties with phylogenetic assignments.[12] Knowing which specific organisms are present on a spacecraft will allow scientists, engineers, and planetary protection officials to assess whether the bioburden poses a genuine threat to planetary protection. Such information on diversity is essential for making data-based decisions about the implementation of bioburden reduction measures that target particular kinds of organisms that might be present on spacecraft. As a first step to this goal, intensive surveys of clean-room and launch facilities could be conducted to identify resident types of microorganisms.

At present, the most effective means of detecting and identifying microbial diversity focuses on the small subunit ribosomal RNA (16S rRNA) gene to determine molecular phylogeny (Pace et al., 1986; Ward et al., 1992).

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[10]The ADK component plus AMP and ATP produces two molecules of ADP. The PPK in the presence of polyP (a linear polymer of many phosphate residues linked by high-energy phosphanhydride bonds) converts the ADP back to ATP. The excess AMP and polyP drive the ADK and PPK equilibrium toward ADP and ATP formation.

[11]See, for example, <las.perkinelmer.com/catalog/Product.aspx?ProductID=6016941>.

[12]Metagenomic technologies (genomic analysis of an assemblage of microorganisms) are constrained by the ability to prepare representative libraries from low-biomass samples and methods for annotating DNA sequences. In some cases, when very low numbers of cells are present, methods such as rolling circle amplification can be employed to amplify the signal; these methods are robust and are approaching the ultimate limit of single-cell whole genome amplification. The greater challenge may be functional annotation. Recent publications cite the use of metagenomic data such as environmental gene tags (EGTs: Tringe et al., 2005). The EGTs reflect the metabolic potential of an analyzed community, and their analysis involves the binning of sequences into functional metabolic groups from different environments containing a range of microbial communities.

The ribosomal RNA gene sequences (16S and 18S) serve as a proxy for the occurrence of microorganisms from the three domains of life (Bacteria, Archaea, and Eukarya) in a sample because this gene is conserved in all known organisms and thus allows comparison of the relatedness between major phyla or between genera. Because of its slow rate of evolutionary change, it is not useful for differentiating between closely related bacterial strains. Diversity assessments made with molecular phylogenetic techniques rely on the amplification of homologous regions of 16S rRNA genes from an environmental sample using the polymerase chain reaction (see Box 6.1 and Figure 6.1.1), along with the analysis of the resultant sequences either by DNA sequencing or fingerprinting methods such as terminal restriction fragment length polymorphisms (T-RFLPs), denaturing gradient gel electrophoresis (DGGE), or microarrays as described below. The molecular phylogenetic approach can be quite useful for planetary protection because it is sensitive and has the potential of yielding quantitative information on individual groups of microorganisms.

Microorganism Identification by DNA Sequencing

Polymerase chain reaction (PCR) amplification of the 16S rRNA gene from a mixed population sample generates a pool of PCR fragments or amplicons of different base composition or sequence. The sequence of each unique PCR product serves as a molecular signature for a microbial species (Pace et al., 1986). By determining the sequence of each unique PCR product, it is therefore possible to detect members of the microbial community that are identical (or nearly so) to entries in DNA sequence databases without requiring their cultivation in the laboratory and to determine their phylogenetic position by using phylogenetic inference algorithms.[13] DNA sequence analysis has become more rapid and cost-effective because of the use of fluorescent dyes to label products of DNA cycle-sequencing reactions and the development of high-throughput capillary instruments that can resolve the fragments of DNA sequencing reactions into ordered nucleotides. Future instruments have the potential to require even less preparation and to make use of nanotechnology, such as that in the Nanopore Project,[14] which allows the analysis of single nucleic acid molecules using ion channels, an approach that could be useful for working with the low biomass that is found in spacecraft assembly rooms.

The molecular approach has been instrumental in revealing the diversity of and identifying novel microorganisms from a wide range of environments such as thermal springs (Reysenbach et al., 1994), the South Pole (Carpenter et al., 2000), solid ice (Priscu et al., 1998, 1999), and the ocean (DeLong et al., 1994; Béjà et al., 2001) that might otherwise escape detection.[15] However, there are limitations and biases to the molecular approach. For example, not all cells are lysed easily during standard DNA extraction methods (Webster et al., 2003), PCR primers may not detect all organisms present (Baker and Cowan, 2004), and PCR reactions tend to preferentially amplify the genes of the most abundant template. With respect to analyzing sequence data, a sufficient number of nucleotides in an appropriate region of the gene must be analyzed in order to ensure that phylogenetic resolution may be achieved. Finally, it is important that a statistically appropriate number of sequences are screened from any given environment or sample to ensure that the diversity is appropriately surveyed (Altekruse et al., 2003; Hughes et al., 2001).

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[13]Phylogentic analyses use statistical procedures and models of mutation to estimate the evolutionary history of DNA sequences. However, different models and inference techniques can yield conflicting evolutionary reconstructions. For example, the deeper lineages of the Bacteria and the protists are controversial (Woese et al., 2000; Brochier and Philippe, 2002). Another potential artifact is caused by horizontal gene transfer among microorganisms. Because molecular trees reflect the evolutionary history at the sequence level, phylogenetic analyses of genes that have undergone horizontal transfer produce a phylogeny that does represent the history of the rest of the genome (Doolittle et al., 2003).

[14]Nanopore detectors are membranes containing a tiny pore called an ion channel, which are large enough to allow only a single strand of DNA to pass through. A voltage applied across the membrane generates an ionic current and pulls the negatively charged DNA molecules through the pore. When the DNA molecule blocks the opening of the nanopore, it causes a characteristic decrease in the current, allowing discrimination between individual DNA molecules. A computer trained by machine-learning techniques recognizes the signals generated by different DNA molecules. For further details, see <www.cbse.ucsc.edu/nanopore.html>.

[15]For example, for nearly a century, investigators considered pink filamentous tufts in Yellowstone thermal springs to represent dead cells or a precipitate. However, the molecular approach revealed them to be members of the Aquificales, a bacterial group important in thermal springs throughout Earth's biosphere and of particular evolutionary interest because of its deep phylogenetic position in some molecular trees.

BOX 6.1
The Polymerase Chain Reaction

Before a cell can divide, it must make an identical copy of all of the genetic information encoded within its DNA. Each daughter cell must inherit all of the original genes after the process of DNA replication, which is mediated by the enzyme called DNA polymerase. The polymerase chain reaction (PCR) is a technique for amplifying (making many copies of) a defined portion of a DNA molecule by using the same biochemical reactions that cells use to copy their genes before they divide. With DNA polymerase as the catalyst, it is possible to convert one copy of a duplex DNA molecule into two copies identical to the first. Each time this process is repeated, the number of copies is doubled. In this way, two cycles generate 4 duplex DNA molecules, three cycles produce 8, four cycles produces 16 duplex molecules, and so on. This exponential increase can rapidly produce billions of copies of the original target genes, making the DNA relatively easy to detect in the laboratory. The process is called a chain reaction because the product of each reaction is more molecules that themselves serve as the substrates (templates) from which more reactions can occur.

The process of PCR amplification is outlined below and shown in Figure 6.1.1.

1. Genes are present in double-stranded DNA complementary to each other. The strands have directionality in opposite orientations. Each strand can be thought of as a template for producing the other, which is why they are called complementary.
2. Before double-stranded DNA can be replicated, the two strands must be separated from one another; in PCR, this is accomplished by heating the reaction mixture to the denaturing point 94°C.
3. Given a single strand of DNA, DNA polymerase catalyzes the synthesis of a new complementary strand, so that the product is a double-stranded DNA identical to the original gene.
4. However, DNA polymerase cannot by itself synthesize the entire second strand; it must start at a "primer," a short single strand of DNA corresponding to the beginning of the "missing" strand. By annealing two specific primers for the reaction (one for each of the two strands) at approximately 52°C, it is possible to define the precise part of the DNA that will be replicated.
5. The process can be repeated many times to produce million- or billion-fold amplification of the desired DNA region (*e* to *g* in Figure 6.1). However, heating the double-stranded DNA molecules for the next cycle of replication (Figure 6.1*e*) can destroy the DNA polymerase from bacteria adapted to grow at temperatures below 38°C, such as *E. coli*. The incorporation of heat-stable DNA polymerases from microorganisms adapted to grow at 72 to 100°C, such as *Thermus aquaticus* and *Pyrococcus furiosus*, into the PCR, permits the amplification process to be repeated without adding any new components.

SOURCE: Modified from NRC (2002), pp. 16-17.

Microorganism Identification by Reverse-Transcriptase (RT)-PCR

RT-PCR employs the same amplification method as does PCR but targets RNA instead of DNA by reverse transcription (coding of an RNA molecule back to its DNA sequence). Because RNA is an essential component of active or metabolizing cells, it is indicative of live cells, whereas amplification of DNA may detect both active and inactive cells. However, this technology is expensive, and it still does not provide quantitative information about relative numbers of different kinds of organisms. It can also fail to identify minor members of the population.

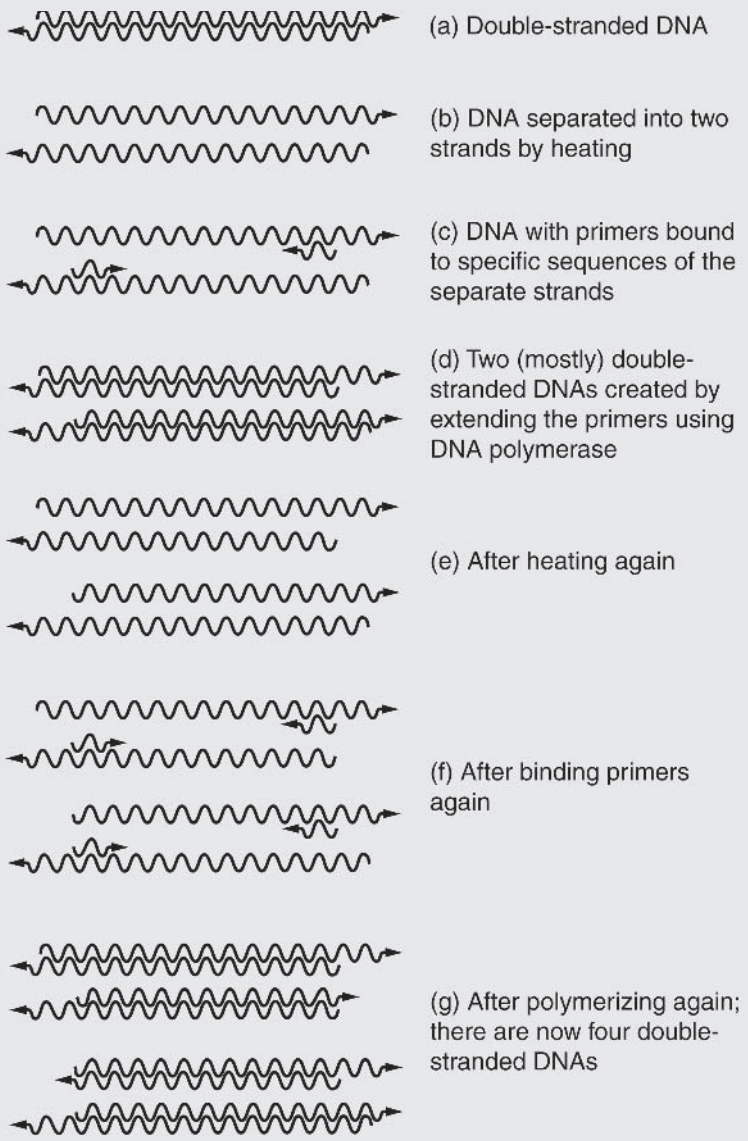

FIGURE 6.1.1 Schematic representation of the successive splitting and copying of nucleic acid molecules in the polymerase chain reaction. SOURCE: Reprinted from Mullis and Faloona (1987), copyright 1987, with permission from Elsevier.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) Analysis

T-RFLP analysis is a rapid method that is easily applied to detect differences in community diversity among samples. The rRNA gene is amplified from genomic DNA and is labeled with a fluorescent dye. The products are then digested with restriction enzymes that recognize and cleave specific short DNA sequences. The fragments are then separated by gel electrophoresis using an automated sequencer. A charge-coupled device (CCD) camera detects the fluorescently labeled fragments; the data resulting from a single sample resemble a chromatograph in

that different phylotypes (sequence types) appear as separate peaks in the electropherogram (output from the sequencing instrument). This method suffers regarding reproducibility, and phylogenetically distinct peaks may be difficult to resolve, yielding no return of sequence data. However, if nucleic acid extraction and PCR amplification methods are standardized across samples, this method can be used to monitor the relative abundance of individual phylotypes, and large numbers of samples can be quickly analyzed. Additionally, individual peaks can be identified by using sequence data developed for the clean rooms.

Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a highly sensitive and rapid technique often used to detect temporal and spatial shifts in microbial diversity. DNA fragments amplified from samples by PCR are separated on an acrylamide gel containing a gradient of denaturant according to base sequence differences. As the fragments migrate through a formamide/urea gradient gel, they melt at different points on the gel corresponding to their sequence.[16] DGGE banding patterns provide a representation of the diversity of the sample, with each band representing a different phylotype. Differences in banding patterns among different samples reflect diversity differences that may result from temporal or spatial changes in community structure. DGGE methods are able to display subtle differences between complex microbial populations, but the assignment of any particular amplicon in a band to a phylotype still requires the determination of that amplicon's DNA sequence. The use of DGGE is less labor intensive than traditional cloning, and it enables rapid estimation of diversity.

Real-Time Polymerase Chain Reaction

Real-time PCR is a method that allows for the enumeration of target gene copy number, a proxy for relative abundance of populations. PCR is performed with fluorescently labeled primers that enable the quantification of amplified PCR products using a CCD camera. The use of primers specific for particular groups of interest enables the comparison of relative population abundances. This method may be especially useful in evaluating the ability of microorganisms identified in clean rooms to survive and grow on Mars.

DNA Microarrays

Printed DNA microarrays consist of glass slides containing thousands of immobilized microscopic samples (probes), each containing oligonucleotide probes targeting the 16S rDNA (or other genes) of specific groups, genera, or species. Fluorescently labeled target DNA is loaded into the wells and any matching strands of DNA will bond to the microarray probes. Positive matches are detected using a fluorescent microscope, a CCD camera, a computer, and the appropriate image analysis software. A single printed slide can contain thousands of probes and can be reused more than 30 times, and the fluorescent dye reporter system can detect 10 attomoles (10^{-18} mole) of DNA but can only detect ribosomal RNAs that are complementary to probes on the array. Similar experimental strategies can be applied to "DNA chips" generated by lithographic processes that produce high densities of microscopic probes with defined oligonucleotide sequences on a matrix.[17]

Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry

DNA and RNA methods are extremely effective at detecting organisms, are not cost prohibitive, and are becoming more rapid. However, there are situations in which these methods fail to resolve individual strains and populations. In the future, if particular organisms are determined to have the potential to colonize Mars, alternative

[16]A denaturant is a substance such as formamide or urea that causes the two strands of DNA to separate or "melt."

[17]It is important to note, however, that various microarray platforms should be evaluated and that probe design, sensitivity, rate of false positives, and cost and reuse issues should be investigated on a case-by-case basis.

methods of detecting target organisms may be needed. Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOFMS) is capable of rapidly detecting and identifying microbial species based on the protein profiles of individual species, and it is effective for both vegetative cells and spores. This method is well suited to the clean room and to identifying microbes on spacecraft because of its rapid throughput, high sensitivity, resolving power, and reproducibility. The drawbacks to this technology include cost and the need to create a database that includes protein profiles for all organisms of interest, a requirement that cannot be achieved without the use of pure cultures. Therefore, this method would not be useful until the microbial diversity of assembly rooms and spacecraft is thoroughly assessed.

None of these advanced detection methods is totally acceptable as a comprehensive measure of biomass and diversity, but neither are spore counts an acceptable proxy for biomass nor an indicator for which organisms may remain on spacecraft after cleaning and sterilization. Applying a combination of methods would offer significant improvements over current approaches to estimating the total bioburden and biodiversity of microorganisms on spacecraft and in the clean room. Moreover, many of the methods described above provide very rapid analysis of total bioburden, which could facilitate spacecraft development and assembly.

METHODS FOR REDUCING BIOBURDEN

Current Approaches and Their Limitations

The current approach to mission design is one in which spacecraft are constructed using conventional materials. This approach is in contrast to the approach taken for Viking missions, whereby materials and components had to be qualified to withstand dry-heat sterilization (see Chapter 2). Currently, spacecraft components and parts are not prequalified in terms of any bioburden reduction methods. Consequently, when a particular sterilization or disinfection procedure is selected, a major test is to determine its effect on spacecraft materials. Issues related to the compatibility of these materials are not typically considered in the hardware engineer's initial model of design and should be determined subsequently. However, hardware can be designed for sterilization, albeit at greater cost, as long as the problem is approached at the onset of design. Incorporating planetary protection into future Mars mission programs at the earliest stages of design would give engineers a selection of effective bioburden reduction tools.[18] The design phase of the Viking mission considered planetary protection requirements and supported investigations of heat sterilization to reduce total bioburden.

The scientific basis for planetary protection in the 1970s continues to guide the implementation of planetary protection protocols despite advances in technology and a dramatically expanded view of microbial diversity. The Viking 1 and 2 spacecraft were dry-heat sterilized at 111.7°C for 29.5 and 23.1 h, respectively (DeVincenzi et al., 1998), whereas spacecraft parts and systems were prequalified at 135°C. The sterilization cycle was based on bioburden determination and lethality correction at all temperatures, not just 111.7°C. That is, there are times when the temperatures increase but have not reached the maximum temperature and subsequently decrease after the maximum temperature has been reached. Document NPR 8020.12C (NASA, 2005) details these requirements.

The highest heat-resistance values for spores detected on spacecraft located in their final assembly and testing environments were used to design the sterilization cycle. Previous research for the Viking program showed that naturally occurring bacterial spores on spacecraft exhibit nonlogarithmic dry-heat inactivation (i.e., the survival curves on semilog graphs are polyphasic rather than linear). This situation reflects mixed populations of different spore formers that display different heat survival curves. These results would be expected, because naturally occurring spore populations are composed of organisms with a wide range of heat resistance. At one end of this spectrum were the least-resistant spore populations that were present in the highest numbers but were killed in the least amount of time. At the other end were the most-resistant spore populations that were present in relatively low numbers but survived for longer periods (Favero, 2004; Bond et al., 1970, 1971; Puleo et al., 1975).

[18]JPL has proposed an architecture study that is considering planetary protection requirements in the architecture, design, manufacturing, assembly, and testing of spacecraft. The study will also consider the vulnerability of spacecraft (components) to Viking-level heat sterilization and how to address this and other planetary protection factors at a system architecture level.

Planetary protection has focused on heat-resistant, spore-forming bacteria, but it is just as important to consider other kinds of microbes. For example, the extremely radiation- and desiccation-resistant bacterium *Deinococcus radiodurans* may be capable of surviving space travel (Clark et al., 1999). Organisms representative of the community found on spacecraft and in clean rooms could be used in the future to validate estimates of bioburden on spacecraft. In addition, the potential for psychrophiles to grow on Mars is at least as likely as that for heat-resistant organisms or thermophiles. There are potential thermal environments on Mars, but the martian near-surface environments (see Chapter 4) are most likely compatible with psychrophiles and psychrotrophs (cold-loving and cold-tolerant organisms, respectively). However, little is known about the sensitivity of these specific organisms to heat treatment.[19] It is possible that the high temperatures used to sterilize the Viking are not necessary, but that cannot be determined without knowing which organisms are present in clean rooms and on spacecraft, and their response to different heat treatments. It is possible that lower temperatures may be effective in reducing the viability of bioburden capable of growing on Mars and that more materials will thus be compatible with bioburden reduction.

The approach to spacecraft sterilization employed by the Viking program was to prequalify spacecraft hardware for dry-heat sterilization. Current bioburden reduction programs for spacecraft use no prequalification criteria for the selection of hardware, due to the added cost to the mission of conducting prequalification studies. Consequently, any bioburden reduction techniques beyond physical cleaning and environmental control should be evaluated to determine materials compatibility issues. When Viking-level sterilization is required, various sterilization procedures should be assessed for use to sterilize spacecraft or specific spacecraft components or systems.

Alternative Methods for Bioburden Reduction

The Viking spacecraft was sterilized by using dry heat. The components and parts of the spacecraft were screened and prequalified to withstand the dry-heat sterilization cycle—an effective but expensive approach. Recent spacecraft destined for Mars have faced a less stringent requirement than did the Viking spacecraft, and physical methods of cleaning (e.g., alcohol wipes) have been considered adequate to reduce the bioburden on the spacecraft to specified levels. However, if physical cleaning alone is not adequate to achieve certain bioburden reduction requirements, alternate methods need to be considered for use on spacecraft. A number of such methods are summarized below:

Heat Sterilization

Steam Sterilization. This procedure, one of the most effective sterilization methods, is used universally in industry, research, and hospitals. Its high moisture and heat content, however, is not compatible with spacecraft hardware.

Dry-Heat Sterilization. This effective sterilization method has been used to sterilize spacecraft. Its main disadvantage is that spacecraft must use hardware compatible with dry heat.

Radiation Sterilization

Electron-beam and gamma radiation are used for sterilization in the medical device industry. Both have the advantage of achieving sterilization at relatively low temperatures, and both penetrate packaging materials, have rapid cycles, leave no residuals (except for dead cells), and have a broad spectrum of microbiocidal activity (i.e., they can kill all types of microorganisms, including bacterial spores). Their disadvantages include the high cost of start-up, safety concerns, materials compatibility issues (including the possible production of ozone), and a requirement for specially trained staff. In the context of spacecraft sterilization, electron-beam sterilization would

[19]The committee recognizes that new discoveries at Mars, for example, evidence for active hydrothermal systems, could change this conclusion and broaden the class of microorganisms of greatest concern.

be a more feasible approach than gamma sterilization, because gamma sterilizers approach the size of a small building, whereas electron-beam sterilizers can fit in a typical laboratory room.

Ambient-Temperature Sterilization

Ethylene Oxide (EO) Sterilization. This method was developed in the 1960s to sterilize instruments and medical devices that were heat labile and could not be steam sterilized. EO is a very effective sterilization system that can sterilize at ambient temperatures, is penetrating, and has a broad microbiocidal range of activity. However, EO is toxic, and there are significant safety concerns. It is used primarily by the medical device industry but much less in hospitals in recent years (Rutala, 1987; Favero and Bond, 2001). The Soviets proposed using EO and methyl bromide for terminal sterilization of their Mars 2 and 3 landers (Hall, 1972; Vashkov et al., 1971).

Newer Methods. A number of new ambient-temperature sterilization systems have been developed in the past decade. These include:

Hydrogen Peroxide Vapor. Hydrogen peroxide in the vapor phase is used commercially for the sterilization of glove boxes and pass-throughs, as well as for decontamination of laboratory incubators and laminar flow cabinets. Hydrogen peroxide is an oxidizing agent that accomplishes sterilization by oxidation of key cellular components. It is a bactericidal, virucidal, sporicidal, and fungicidal agent, even at low concentration and temperature.

Hydrogen Peroxide Gas Plasma. This process uses hydrogen peroxide in the vapor phase and low-temperature gas plasma for rapid inactivation of microorganisms and the removal of harmful residues. Gas plasmas are highly ionized gases composed of ions electrons, and neutral particles. A solution of hydrogen peroxide and water is vaporized and allowed to surround and interact with the devices to be sterilized. Applying a strong electrical field then creates plasma. The plasma converts hydrogen peroxide into water and oxygen, and there is thus no hydrogen peroxide residual left on sterilized loads and no hydrogen peroxide released into the environment nor exposure of technical staff. No aeration time is required, and the sterilized materials can be used immediately. This method is relatively rapid; for example, a load of surgical instruments can be sterilized in less than 1 h (Jacobs and Lin, 2001).

Ozone. Ozone is produced by passing dry air or oxygen between high-voltage electrodes that produce a coronal discharge, or by UV irradiation of air or oxygen. Because of its instability, the gas has to be produced at the point of use. Ozone is triatomic oxygen with one loosely bonded O atom; this readily attaches to other molecules and makes ozone a powerful oxidizing agent. It is a bactericidal, virucidal, sporicidal, and fungicidal agent and cycles between 4 to 5 h. Currently, a low-temperature sterilizer is sold in the United States and Canada. The sterilizer has been approved by the U.S. Food and Drug Administration for sale and is used to sterilize medical instruments and devices (Josyln, 2001).

Chlorine Dioxide. Chlorine dioxide in its gaseous form (CD) is approved for use as a sterilant by the U.S. Environmental Protection Agency. Its sporicidal effects are well documented and can be compared to those of vapor-phase hydrogen peroxide and hydrogen peroxide gas plasma. CD is not prepared by the vaporization of solution but is actually generated at the point of use. This is accomplished by using a method in which solid sodium chlorite contained in small plastic cartridges is contacted by a gas mixture of 2 percent chlorine and 98 percent nitrogen. The reaction products are CD and sodium chloride. CD is used for the sterilization of medical products and glove boxes and for the decontamination of small areas.

Liquid Chemical Disinfection

A number of liquid chemical germicides formulated as sterilants and high-level disinfectants are used for reprocessing of instruments and medical devices. They include formulations containing glutaraldehyde, ortho-phthalaldehyde, hydrogen peroxide, peracetic acid, and combinations of these. They are effective biocides and can

be used at low temperatures. In the context of spacecraft sterilization, the main disadvantages are materials incompatibility with oxidative formulations and the fact that they are in liquid form.

Encapsulated Bioburden

Levels of microbial contamination within solid materials, encapsulated components, occluded surfaces, lubricants, or surface finishes of Mars spacecraft have always been of concern. However, there is no published research investigating the actual bioburden contained within materials or its potential to survive if released (e.g., during a crash) because of the difficulty in assaying these regions (see Chapter 2). Furthermore, current estimates in use (see NPR 8020.12C; NASA, 2005, pp. 39-41) were developed for spacecraft materials and components 30 years ago and have not been updated.

Historically, mission engineers have worked hard to reduce accessible bioburden (e.g., on the surface of the spacecraft) to compensate for the poorly known encapsulated bioburden in the total bioburden estimate. Still, methods have not been developed for determining concentrations of bioburden encapsulated in spacecraft components, and little attention has been paid to improving understanding of this potential microbial reservoir. New methods should measure the true level of viable contamination in encapsulated and mated surfaces of contemporary and future spacecraft materials, and models should be developed for extrapolating bioburden levels to components that cannot be directly assayed.

SUMMARY

The use of swab cultures to test for spores (see Appendix C) has been a valuable approach for determining bioburdens on spacecraft, but it is not without limitations. Recent advances in microbial ecology argue that spores are an imperfect proxy for estimating microbial bioburden, and culturing methods do not provide a representative estimation of total bioburden on pre-launch spacecraft or in assembly areas. It is of great importance to know both the amounts of bioburden remaining on a spacecraft and the kinds of organisms that are present. As described in the section above titled "Biomarker Methods," the ATP and LAL assays are promising emerging technologies that could offer rapid estimates of bioburden without the need for laboratory culturing. In addition, when the organisms present have been identified, cleaning and sterilization can be focused more specifically on organisms of importance, that is, those that have the potential to survive flight to Mars and then grow in the martian environment, rather than on spores per se. The committee recognizes that, given rapidly changing knowledge of potential special regions on Mars (see Chapter 4), no class of microorganisms can currently be identified with complete confidence as being that of greatest importance for preventing the forward contamination of Mars. This situation emphasizes the need to understand the full diversity of organisms on spacecraft and to adapt bioburden assessment and reduction methods to new planetary and biological knowledge. A number of rapid methods exist that will enable for more direct estimation and identification of viable cells, as well as many new methods of bioburden reduction and sterilization that could allow mission engineers a suite of materials with which to design spacecraft. The next step is to assess these methods with respect to NASA planetary protection guidelines, evaluate their efficacy in comparison to current spore and culture methods, determine the optimal methodological approach for updating planetary protection requirements, and develop a defined standard certification process that will allow engineers and planetary protection officers to implement these advances as early in the mission development process as possible.

Ultimately, the use of advanced detection methods to identify the potential bioburden may lead to bioburden reduction techniques that are targeted at groups of organisms of specific concern for planetary protection. NASA has been investigating some of these technologies, including LAL and ATP analysis (e.g., Dickinson et al., 2004a,b; Venkateswaran et al., 2001, 2003), and research on bioburden reduction methods has also been proceeding.[20] However, additional research on these technologies is needed. At the very least, such research will provide the data needed to determine what future mix of methods is appropriate to minimize the forward contamination of Mars.

[20]See footnote 2.

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7

Assessing Nonliving Contaminants of Concern

Technological developments are leading to increasingly sensitive and sophisticated instruments that may be applied to the search for life on Mars (see Chapter 6). As a result, the scope of the search has expanded to encompass signs of past as well as extant life, and the issues that could complicate or compromise the search have increased accordingly.

Planetary protection of Mars has previously emphasized the need to restrict a spacecraft's burden of living organisms to prevent biological contamination of Mars and to avoid jeopardizing experiments designed to detect life there. But because many of the newer techniques for detection of life depend on the measurement of trace quantities of specific molecules, it is important that planetary protection measures also address the level of nonliving contamination of spacecraft that could confound with false positives the results obtained with such techniques.

The issue of contamination of Mars from nonliving sources was noted briefly by the NRC in 1992 (NRC, 1992, pp. 38-39). More recently, the challenges posed to life-detection experiments by nonliving contaminants were addressed by the Organic Contamination Science Steering Group (OCSSG) in a report (Mahaffy et al., 2003) that greatly aided the deliberations of the Committee on Preventing the Forward Contamination of Mars.[1]

This chapter describes six categories of nonliving materials as potential contaminants that could compromise the search for extant or extinct life on Mars. Examples of such contaminants introduced by spacecraft to Mars include organic molecules derived from living and nonliving matter, nutrient elements such as nitrogen, and particulates (dust). At worst, such nonliving contaminants potentially could (1) confound the detection of life by instruments carried on a particular mission to Mars, (2) contribute to the contamination of particular regions of interest that could complicate future life detection there, (3) promote or assist the growth of contaminating Earth microbes transferred concurrently or previously by spacecraft, and (4) compromise measurements of Mars atmosphere trace gases of significance for life detection.

[1]The OCSSG report was chartered by NASA's Mars Program Office following the recommendations of the Mars Exploration Program Analysis Group (MEPAG) at its September 2003 meeting (MEPAG, 2003).

TYPES OF CONTAMINANTS

All Earth materials, from atoms to molecules to organisms to the actual spacecraft and landers themselves, could be considered contaminants on Mars in specific contexts. Thus it is necessary to define the nature and the threshold levels of materials of concern for planetary protection—specifically with reference to the science objectives of a particular mission to Mars and to the analytical sensitivities of present-generation instruments, as well as in anticipation of not compromising subsequent missions. At least six categories of materials, biological and nonbiological, as well as organic[2] and nonorganic materials should be considered explicitly for their potential to contaminate Mars. They are considered briefly below. The OCSSG report (Mahaffy et al., 2003) addresses organic contaminants in more detail than can be presented here.

Compounds Derived from Biological Sources

Substances derived from biological sources could potentially compromise current or future efforts to detect life on Mars, since many of the techniques used for that purpose work at the molecular level to determine the presence of biomolecules and biological activity (see Chapter 6). Such biological compounds include DNA and individual nucleotides, proteins and individual amino acids, complex lipids, complex carbohydrates, and energy carriers such as adenosine triphosphate (ATP). All such compounds, if present as contaminants, could result in a false-positive result interpreted as signaling the presence of living cells in the present-day Mars environment—and could thus compromise the ability of researchers to evaluate the outcomes of scientific experiments concerned with detection of life on Mars.

Materials That Could Serve as Substrates for Microbial Metabolism

Contamination of Mars by Earth microbes could be aided by the introduction to Mars of sources of carbon and energy-yielding substrates. The transfer to Mars of such materials could change the local Mars environment and hence its habitability for microorganisms. Microbes on Earth display an astonishing diversity of metabolic capability (see Chapter 5); environments have been observed and species isolated in which microbial growth occurs anaerobically by the oxidation of unusual substrates such as benzene, trinitro-toluene (TNT), trichloro-ethylene, and numerous other solvents, plastics, and explosives, provided that water is also present (e.g., Lovley et al., 1994; Bradley and Chapelle, 1996; Esteve-Nuñez et al., 2000). Solvents may be present on spacecraft as residues from cleaning procedures; hydrocarbons may be used as lubricants for mechanical components; plastics, including Teflon, Kevlar, and other composites, are used as structural components and in instruments; and benzene and other polyaromatic compounds are formed during the thermal breakdown of other carbon residues. In the case of microbial oxidation of these reduced organic materials, ferric iron [Fe(III)] is frequently the oxidant. On Mars, contaminant organic materials could be exposed to abundant oxidizing power in the form of ferric minerals, including nonspecific iron oxides and hematite crystals (Morris et al., 2004). Preventing the development of "microbial islands" or growth pockets aboard spacecraft and/or landers will depend in part on the ability to limit the contact between microbial cells, organic substrates, oxidants such as Fe(III), and water.

Light Elements Critical for Microbial Growth

In addition to carbon, energy sources, and water, nutrients critical for growth include nitrogen, phosphorus, and sulfur. Also included in this category are the trace elements selenium, molybdenum, copper, zinc, iron, cobalt, and nickel. Although it is unlikely that future Mars science missions will rely strictly on the analysis of elements to determine biosignatures, it is the case that excessive contamination by these elements could expedite the growth

[2]An organic material is one that includes carbon covalently bonded with hydrogen and perhaps other atoms. Therefore, most carbon-based molecules are organic molecules, but CO, CO_2, and certain others are not, despite their potential importance in the biochemistry of life.

of co-transported terrestrial organisms. With the exception of nitrogen, all of these elements are transmissible primarily as particulate material or as dissolved ions. Salts, dust, aerosols, and fingerprints are all of concern for the control of contamination, both on the surfaces and within the interior spaces of spacecraft, as well as for the potential transfer of contaminants from spacecraft components to Mars. Nitrogen as a contaminant could take many forms in addition to particulate matter: it could also be present in nitrogenous salts and in volatile phases, including as N_2 gas; the reduced species ammonia, hydrazine, and cyanide; and the oxidized forms N_2O and nitrogen oxides (NO_x). Nitrogen and phosphorus are fundamental nutrient elements critical for the growth of all organisms; thus, they are potential contaminants to be avoided.

Simple Organic Molecules

Simple organic molecules include formaldehyde, cyanide, acetate, urea, simple sugars, amino acids, benzene, polycyclic aromatic hydrocarbons (PAHs), and light hydrocarbons. In addition to serving as potential substrates for microbial growth and as substrates for the formation of more complex macromolecules, these compounds are generated by organisms and are potential indicators of the presence of life. Contamination of Mars by exogenous material could compromise the ability to make the necessary distinctions; sources of such exogenous material could include the inevitable meteoritic input but could also be greatly enhanced locally by contamination from spacecraft.

Simple organic molecules can be produced through the breakdown of substances of biological or synthetic origin. Such materials are both commonplace in and problematic for life-detection experiments, because their high volatility can lead to their migration to surfaces previously uncontaminated by these molecules. The recoating of previously cleaned surfaces exposed to volatilized and migrating organic molecules requires diligent monitoring. As recommended in the OCSSG report (Mahaffy et al., 2003), approaches to monitoring the migration of contaminants throughout a spacecraft include the use of witness plates, development of transport models appropriate to the atmospheric pressure of Mars, and maintenance of an archive of all organic-containing materials used during spacecraft assembly.

Polymers

Biopolymers include the polysaccharides, proteins, lipids, and polynucleotides mentioned above, as well as lignin, chitin (amino-sugar polymer), and humin, all of which are found in terrestrial soils and therefore are possible components of dusts incorporated into spacecraft during assembly. Synthetic polymers include the plastics, plastic residues, and Teflon; they are typically present as spacecraft and payload component materials. Other nonvolatile complex molecules in this category include pigments and porphyrins (biological) and epoxies and adhesives (usually synthetic). Biopolymers that could compromise searches for life might be targeted for removal by spacecraft cleaning (Mahaffy et al., 2003).

Propulsion Exhaust Products of Space Vehicles

Materials in the five categories of potential Mars contaminants outlined above all release trace amounts of matter through, for example, outgassing, migration of microbial degradation products, and incidental contact. A sixth category of materials involves the exhaust products of Mars lander or vehicle propulsion systems, as well as exhaust intentionally released onto the martian surface and into the atmosphere. Little research has been conducted to assess the potential for such contamination to confound future scientific investigations. The concern about such contamination will grow, as the number and size of lander missions is expected to increase as the Mars Exploration Program evolves.[3]

[3]The committee is not aware of any detailed analysis regarding potential contamination by spacecraft propellants released in a crash of a space vehicle.

Any Mars mission that enters the martian atmosphere and reaches the surface will emit solid, liquid, or gaseous nonbiological materials that are foreign to the Mars environment, including propulsion exhaust products in amounts ranging from as little as a few kilograms (as was the case for Pathfinder) to as much as 30 metric tons for a Mars excursion vehicle with a crew of four people and a surface payload of 25 metric tons (MSFC, 1991). Two concerns arise: (1) Could contamination caused by these exhaust products confound the measurement capabilities of the mission's science payload? (2) Could such contamination affect the global environment so as to alter or mask important atmospheric markers of past or current conditions potentially relevant to biological investigation of Mars?

Since the Viking missions in the mid-1970s, Mars missions with soft landers (landers that touch down on the surface at speeds below a few meters per second) have used purified hydrazine fuel for the terminal descent phase (Husted et al., 1977). Use of purified hydrazine avoids contamination of the landing site with water and assorted carbon compounds, because the primary exhaust products are hydrogen, nitrogen, and ammonia. (See also Appendix G, Table G.1.) Other landers using passive devices such as airbags to absorb surface impact at higher landing speeds (e.g., Pathfinder and the Mars Exploration Rovers (MER) Spirit and Opportunity) may use solid retrorockets to more crudely control their terminal velocity. Solid rocket plumes contain aluminum oxide and carbonaceous compounds arising from pyrolysis of the ethylene propylene diene monomer rubber case insulation. To date, these solid rocket plume exhaust products have not been assessed as possible contaminants because it has been assumed that any impact they might have on the environment would occur well away from the final resting place of the landers.

Future mission concepts such as Mars Sample Return (MSR), Mars airplanes, and human missions entail more landed mass and/or higher total mission impulse (i.e., energy dissipation). These missions imply the use of propellants with higher energy content, and the exhaust products from this class of propellants usually contain measurable amounts of water and various other compounds, including carbon monoxide and carbon dioxide. One example of this type of propulsion is the first stage of the Mars Ascent Vehicle (MAV) for the planned MSR. Current designs for MSR use a solid motor first stage that ignites at the surface and burns to an altitude of approximately 15 km, emitting tens of kilograms of H_2O, N_2, CO, HCl, and Al_2O_3. Whether or not such contamination is tolerable, given that the martian samples to be returned will already have been collected, will depend in part on any subsequent surface investigations planned after the ascent stage launch, as well as the nature of the atmospheric contamination from the solid motor first stage occurring during launch. These issues are considered in greater detail in Appendix G.

DETERMINATION OF ACCEPTABLE LEVELS OF CONTAMINATION

Determining the background level allowable for each of the foregoing categories of materials requires an evaluation of the analytical limits of detection of current instruments, as well as of improvements that may be expected in future generations of instruments. The OCSSG report (Mahaffy et al., 2003) addresses these concerns with respect to mass fraction, dividing categories of molecules into components of high concern (1 to 100 ppb threshold) or medium to low concern (>100 ppb to ppm threshold).[4] The implication of such mass-based standards is the requirement that a scientifically targeted molecule of "high concern" would have to be detected in a sample at a threshold of >100 ppb to be considered relevant. Simultaneously, it would be established or assumed that the contamination background was substantially <100 ppb. Such standards represent challenges both for the development of monitoring efforts and for the selection of appropriate instrument technologies.

Quality control for the detection and monitoring of contamination levels can be achieved in at least four ways:

1. *Monitoring of the spacecraft and facility throughout assembly.* Witness plates, Teflon swabs, and alcohol wipes can be used both to maintain cleanliness (see Appendix C) and to assess the mass and composition of residues removed from the spacecraft and instruments (Mahaffy et al., 2003).

[4]See Tables 2 and 3 and Appendix A in the OCSSG report (Mahaffy et al., 2003).

2. *Monitoring of volatiles during bakeout.* Volatile compounds can be monitored during a thermal bakeout procedure in which spacecraft components are heated to release volatile compounds and thus reduce the rate of escape and migration of such volatiles during the mission (Mahaffy et al., 2003). This outgassing of volatiles can be monitored and quantified by sample collection and identification of the volatiles. Models generated from these data can be used to predict the behavior of volatile compounds in ambient Mars conditions and assess whether a given material will cause an unacceptable background level of either total carbon or individual compounds.

3. *Incorporation of "blanks" into the instrument payload.* Use of blanks—carefully selected control materials (e.g., mineral samples) that are free of an analyte of interest (e.g., individual amino acids)—can help in the determination of background levels of contamination acquired throughout the launch, transit, and landing process of a lander deployed to Mars. This process requires that the standard appropriately mimic the texture and structure of the Mars samples to be analyzed but that it contain levels of the target analyte that are lower than can be detected with existing technology. The use of blanks could be particularly effective for verifying the authenticity of martian organics; however, the use of blanks could be difficult to implement in a practical manner, possibly requiring planning and preparation by the scientific community of a set of standards applicable to the anticipated wide range of scientific experiments associated with any particular mission.

4. *Use of terrestrial standards.* Standards containing rigorously calibrated concentrations of potential analytes of interest also could be included with a payload to Mars to support accurate determination of the concentrations of analytes found in Mars samples. The benefits of such a technique include the ability to recalibrate instruments and achieve greater analytical precision and accuracy in the analysis of Mars samples. However, as noted in the OCSSG report (Mahaffy et al., 2003), the potential contamination hazards associated with transporting these standard materials on spacecraft appears to be significant. If materials deliberately containing above-threshold levels are transported and analyzed in situ, accidents or equipment failures potentially could allow for insurmountable contamination of both the instruments and the martian sample environment.

SUMMARY

The assessment of nonliving contaminants of concern is complex. The materials identified in this chapter are likely to be transferred to Mars by different processes and at different mass scales; they have varying potential to compromise experiments designed to detect life on Mars; and the acceptable levels of contamination may have different thresholds depending on the class of contaminant, as well as on the scientific demands of individual Mars missions. Preventing the microbial contamination of Mars, however, includes consideration of such nonliving material. In the presence of living cells and liquid water—either on a spacecraft or transferred to the Mars surface environment—several classes of organic and inorganic compounds could contribute to the growth of microbes and limit the ability to accurately detect their presence. This includes the transfer of labile biomolecules, both monomeric (e.g., nucleotides, amino acids, sugars, acetic acid, and fatty acids) and polymeric (DNA, proteins, polysaccharides, lipids). It also includes the potential to contaminate Mars with the most critical nutrient elements—nitrogen and phosphorus. Other organic contaminants such as polymers, plasticizers, and combustion products (PAHs) may be less likely to confound life-detection experiments, but they are ubiquitous and correspondingly harder to avoid introducing as contaminants. Finally, on a total mass basis, contamination of the Mars atmosphere by the exhaust products of rocket propellants is possibly the greatest source of exogenous material. However, it also is less clear to what extent these gases might be interpreted as indicators of biological processes.

Clearly, for all the reasons presented, comprehensive consideration of nonliving contaminants should be an integral part of spacecraft cleanliness requirements and the processes implemented in mission development and flight to comply with these requirements.

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8

A Path Forward for Planetary Protection in the 21st Century

Increased scientific understanding of the martian environment (see Chapter 4) and the ability of microorganisms to survive in severe conditions (see Chapter 5) have important implications for the planetary protection of Mars. Ongoing missions such as Mars Global Surveyor, Mars Odyssey, Mars Express, and Spirit and Opportunity, as well as continuing ground-based observations, are producing a rolling wave of scientific discoveries about Mars (see Chapter 3). Anticipated Mars missions will likely travel to locations with greater potential for the survival and possibly the growth of Earth microbes. The science and engineering community needs to ensure, on an ongoing basis, that planetary protection policy and practices reflect current scientific and technical understanding and capabilities.

As a result of its study, the Committee on Preventing the Forward Contamination of Mars found that (1) many of the existing policies and practices for preventing the forward contamination of Mars are outdated in light of new scientific evidence about Mars and current research on the ability of microorganisms to survive in severe conditions on Earth; (2) a host of research and development efforts are needed to update planetary protection requirements so as to reduce the uncertainties in preventing the forward contamination of Mars; (3) updating planetary protection practices will require additional budgetary, management, and infrastructure support; and (4) updating planetary protection practices will require a roadmap, including a transition plan with interim requirements, and a schedule. In addition, the committee found that scientific data from ongoing Mars missions may point toward the possibility that Mars could have locales that would permit the growth of microbes brought from Earth, or that could even harbor extant life (although this remains unknown),[1] and that these intriguing scientific results raise potentially important questions about protecting the planet Mars itself, in addition to protecting the scientific investigations that might be performed there.

Drawing on information presented in Chapters 1 through 7 of this report, the committee presents below its findings and recommendations for preventing the forward contamination of Mars. The recommendations are organized according to five themes: (1) protection of mission science and protection of the planet, (2) programmatic support, (3) research and reconnaissance, (4) transition to a new approach, and (5) interim requirements. The committee urges that its recommendations be considered as a package; the recommendations build one on the other to establish a new planetary protection framework. Chapter 9 presents a suggested transition plan and process for implementing the committee's recommendations.

[1]See Chapters 4 and 5 and references therein.

EXPANDING THE PURPOSE OF PLANETARY PROTECTION: SAFEGUARDING OF INDIGENOUS LIFE AS WELL AS PROTECTION OF MISSION SCIENCE?

Historically, planetary protection policy has addressed the concern that the forward contamination of planetary environments by terrestrial organisms could compromise spacecraft investigations sent to identify indigenous life.[2] As a result, current practice imposes the strictest standards of cleanliness on those spacecraft that will conduct life-detection experiments. Other spacecraft that will not search for life are required to meet less stringent standards.

Although this policy may succeed in protecting the integrity of Mars mission science during the near-term period of biological exploration, recent discoveries suggest that there may be numerous (and potentially difficult to detect) environments on Mars where the probability of growth for terrestrial organisms is substantially higher than previously thought. If so, there is the potential that the lower standard of cleanliness afforded for spacecraft that do not include life-detection experiments may allow the introduction of terrestrial organisms into sensitive environments where they may reproduce.

The ethical and policy implications of questions about protection of the planet Mars are not currently addressed by either the Outer Space Treaty or COSPAR policies. Although they fall outside the mandate of the current committee, the committee believes that their consideration should have high priority. The need for urgency in deliberations on protection of the planet Mars as well as protection of science is underscored by the present uncertainty about the distribution of such sensitive martian environments, the failure rate and cleanliness levels of Mars landers, and the projected rapid pace of future spacecraft investigations (see Box 8.1). For these reasons, the committee believes that it is important that NASA and its international partners address questions about the protection of the planet Mars as expeditiously as possible.

Recommendation 1. **In light of new knowledge about Mars and the diversity and survivability of terrestrial microorganisms in extreme environments, NASA should work with COSPAR and other appropriate organizations to convene, at the earliest opportunity, an international workshop to consider whether planetary protection policies for Mars should be extended beyond protecting the science to include protecting the planet. This workshop should focus explicitly on (1) ethical implications and the responsibility to explore Mars in a manner that minimizes the harmful impacts of those activities on potential indigenous biospheres (whether suspected or known to be extant), (2) whether revisions to current planetary protection policies are necessary to address this concern, and (3) how to involve the public in such a dialogue about the ethical aspects of planetary protection.**

PROGRAMMATIC SUPPORT

Many existing planetary protection practices stem from the R&D on planetary protection that was conducted during the 1970s in preparation for sending the Viking life-detection missions to Mars. Since that time, knowledge about Earth organisms and their ability to survive under severe conditions has advanced considerably, and the potential presence of such organisms on spacecraft may warrant alternative approaches to reducing bioburden. Over the last 30 years as well, new technologies for assessing microbial diversity and reducing bioburden on spacecraft have been developed. If applied properly, they should allow researchers, engineers, and planetary protection officials to improve both microbial detection and bioburden reduction methods compared to those currently being used (see Chapter 6). Transitioning NASA's planetary protection practices so that they reflect current scientific understanding of Mars and microbiology and also benefit from the use of advanced technologies will require investing in a series of R&D efforts on and assessments of new technologies that can be applied to the implementation of planetary protection policies. It will also require a structure for managing such research efforts in coordination with the engineering, spacecraft and instrument development, and science communities at NASA

[2]This position originated in the decade before the promulgation of the Outer Space Treaty of 1967 (see Chapter 1).

BOX 8.1
Perspectives on the Potential for Contamination of Mars Posed by Past and Future Missions

Many committee members agreed with issues regarding the potential for contamination of the planet Mars posed by past and future missions. Indeed, COSPAR's current planetary protection policy focuses implicitly on protecting the planets for scientific exploration. Over the years, debates about standards of cleanliness for spacecraft have centered on whether the standards are strict enough to ensure the integrity of life-detection efforts during the period of biological exploration (see Chapters 1 and 2). More broadly, it is also uncertain what the long-term fate or effects of terrestrial microbes might be on the potential indigenous biosphere, locally or planetwide.

Since the start of robotic exploration of Mars, 12 spacecraft have landed or crashed on the planet's surface (Table 1.1). Each was cleaned to the bioburden levels deemed appropriate for its individual mission at the time of its launch.[1] Even so, given current recognition of the potential nature, diversity, and distribution of habitable environments on Mars, the adequacy of these cleanliness levels is now questioned. Thus, the committee's recommendations 12 through 14 advise more stringent requirements for bioburden reduction for Mars missions. Much of the uncertainty about the risks of contaminating the martian biosphere involves the survival and growth rates of microbial contaminants that may be present on spacecraft when they arrive.

The problem is compounded when one considers the very long term. Current climate models suggest that, at times of high obliquity ($i > 45°$), summertime surface temperatures at polar and near-polar latitudes may exceed the melting point of water for continuous periods of many months. Such conditions may be repeated annually for perhaps thousands of years (i.e., for as long as the high-obliquity phase of the 10^5-year obliquity cycle continues; see Chapter 4 and Appendix F). The effect is to make these ice-rich, high-latitude environments among the most potentially habitable surface environments on Mars for the survival and growth of terrestrial microbes. In addition to the uncertainty about whether these environments may become habitable in response to future environmental or climatic change, and whether terrestrial microbes introduced to Mars today could survive for 10^4 to 10^5 years until the climate again swings, there is concern about the potential for spacecraft to contribute to possibly irreversible contamination of these environments, despite compliance with planetary protection controls. Not only are these questions scientifically relevant for life-detection and planetary exploration efforts, but they also have potentially major implications for the martian biosphere itself. Ramifications extend into the ethical and philosophical realms, beyond the purview of this committee and current international policies.

Committee perspectives differed on but included the view that upcoming missions demonstrate the urgency with which such ethical concerns should be considered, even if those missions comply with existing planetary protection policies. Some committee members believed that missions that are in compliance with existing policies should not be subjected to further scrutiny. However, others thought it important to discuss missions that could potentially have irreparable effects on a martian biosphere. Two missions were cited as illustrative of this concern. The Mars Polar Lander (MPL) experienced a failure during its final descent in December 1999 and crashed into the ice-rich south polar layered deposits. Because the spacecraft did not include a life-detection experiment, it was cleaned to the Category IVa (Viking pre-sterilization) standard. As a result, surviving pieces of the spacecraft, bearing relatively high bioburdens of terrestrial organisms, may have become embedded in the polar ice. The Phoenix Scout mission (2007) is being sent to investigate a similar high-latitude, ice-rich environment and is being cleaned to approximately the same

continues

[1]Few data on planetary protection measures taken for missions of the former Soviet Union are available. However, NASA memoranda from Lawrence B. Hall, NASA's planetary protection officer in 1972, suggest that the Soviets applied bioburden reduction measures to the Mars 2 and 3 spacecraft, including the use of methyl bromide-ethylene oxide gas. The memoranda include summary translations of Soviet documents describing their practices. Soviet planetary protection measures were judged by Hall to "approximate compliance with COSPAR constraints," assuming that the Soviet program "did, or will, carry out the measures described."

BOX 8.1 Continued

standard. However, those portions of the spacecraft that are expected to come into direct contact with the surface (i.e., its footpads and robotic arm-mounted shovel) are being cleaned to the more stringent Viking post-sterilization (IVb) standard. A significant concern is whether, by accident or nominal operations, such spacecraft could contribute to the irreversible contamination of sensitive environments, despite compliance with current planetary protection controls. In addition to its relevance to life-detection and planetary exploration efforts, this situation has potentially major implications for the long-term health and survival of a martian biosphere, should one exist.

Within the committee, reaction to this concern was considerably varied: at one extreme, there was a call for the immediate review of planetary protection requirements for all missions in development, while at the other extreme, it was held that before the acquisition of additional data, no immediate action should be taken. The majority of the committee members found themselves somewhere between these two views.

headquarters, NASA centers, and in universities, research laboratories, and industry, as well as with the international community.

The committee recognizes that such research efforts have cost implications; however, in the committee's view, the Mars Exploration Program's focus on the search for past and present life (Chapters 1 and 3) requires that additional resources be committed for updating planetary protection practices, and that this be done in a sustainable way that ensures a new generation of scientists and engineers with expertise in this area. Such investment would also introduce innovation that could potentially lead to techniques for planetary protection that are faster, more accurate, and more effective at reducing bioburden on spacecraft bound for Mars, all of which could be cost-effective in the long term.

Recommendation 2. **NASA should establish and budget adequately for, on an ongoing basis, a coordinated research initiative, management capability, and infrastructure to research, develop, and implement improved planetary protection procedures. The research initiative should include a training component to encourage the growth of national expertise relevant to planetary protection.**

Currently, planetary protection often is not emphasized until the spacecraft production process. The committee supports the Jet Propulsion Laboratory's plans to assess spacecraft design and development processes with a view toward considering planetary protection at the earliest stages of a mission project. Bioburden reduction will be most effective and most efficient if it is built into mission planning and design from the earliest stages.

Recommendation 3. **Future missions to Mars should plan for the effective implementation of planetary protection requirements at the earliest stages of mission and instrument design, and engineers should be provided with a selection of effective, certified tools for bioburden reduction.**

NASA's Mars Exploration Program is planning a series of missions of increasing scientific and technological capability that will, inter alia, explore potential martian habitats for life (see Chapter 3). The resulting wave of data may significantly change the scientific understanding of Mars and its environment. Scientific understanding of microorganisms on Earth is also increasing rapidly, and molecular methodology has revealed that more than 99 percent of suspected terrestrial microbial species still remain largely uncharacterized (see Chapter 5). The analysis of these forthcoming data may suggest the need to either relax or increase the stringency of planetary protection requirements for preventing forward contamination. The committee believes that the current pace of exploration and discovery on Mars places unprecedented pressure on the adequacy of planetary protection requirements and protocols, a situation that will require dialogue involving a broad range of scientific viewpoints, ongoing dynamic adaptation, and continuing oversight by all governing and implementing organizations.

Recommendation 4. **NASA should establish an independent review panel that meets every 3 years to (1) consider the latest scientific information about Mars, as well as about Earth microorganisms, and recommend to NASA appropriate modifications to NASA's planetary protection implementation requirements as needed in light of new knowledge; and (2) identify and define the highest-priority measurements needed at Mars to inform future assessments and possible modifications of planetary protection requirements.**

The first meeting of the review panel should be held in 2008. Meetings should occur every 3 years thereafter, unless major changes in understanding of Mars or other factors related to planetary protection require meetings on an urgent basis.

NEEDED RESEARCH AND RECONNAISSANCE

Planetary protection policy and practice should be based on the most up-to-date understanding of the microbial bioburden on and embedded in spacecraft sent to Mars and the kinds of environments relative to life that may be encountered there. Modern means to reduce bioload, in addition to dry-heat sterilization, should be widely available for use in spacecraft and component manufacture. This section summarizes key recommendations intended to ensure that the research necessary to meet these objectives is conducted.

Although recorded data on bioburden levels during assembly, test, and launch operations (ATLO) are sufficient to certify compliance with current requirements for total bioload (densities and numbers), there is little information to indicate the taxonomic diversity and densities of microbes on spacecraft hardware or in clean-room areas. The committee commends NASA's support of the preliminary work in this area (Dickinson et al., 2004a,b; Venkateswaran et al., 2001, 2003).[3]

Information on the diversity and density of microbes on spacecraft hardware could be useful in reconsidering the probability of growth of transported terrestrial microbes, given recent discoveries about martian environmental conditions. In addition, a more complete understanding of actual transported bioburdens would be useful in designing controls to rule out false positives during future life-detection investigations.

Moreover, because molecular techniques are constantly advancing, and because the future may reveal the need for more information about what organisms may have been delivered from Earth to particular sites on Mars, it is important that routine and long-term archiving of environmental samples, as well as phylogenetic data, be adequately supported.

Recommendation 5. **NASA should require the routine collection of phylogenetic data to a statistically appropriate level to ensure that the diversity of microbes in assembly, test, and launch operations (ATLO) environments, and in and on all NASA spacecraft to be sent to Mars, is reliably assessed.[4] NASA should also require the systematic archiving of environmental samples from ATLO environments and from all spacecraft to be sent to Mars.**

On the basis of current knowledge about Mars (see Chapter 4), the committee believes that psychrophilic or psychrotrophic organisms are those most likely to grow in a martian near-surface environment (Chapter 5), although this evaluation may evolve as knowledge of Mars improves.[5] For example, detection of active near-surface hydrothermal vents would expand the class of microorganisms that could grow in the martian near-surface. Recommendation 4 of this report addresses the need to update planetary protection requirements, if necessary, in

[3]As noted in Chapter 6, NASA is currently investigating advanced microbial detection and bioburden reduction methods, but additional research on these technologies is needed.

[4]Analytical models such as rarefaction or Bayesian inference should be used to ensure the completeness of a survey, and a sufficient number of nucleotides per gene sequence should be used to differentiate among unique sequences (e.g., Altekruse et al., 2003).

[5]The committee recognizes that liquid water could also exist in the warm, deep subsurface of Mars and create the conditions for a possible biosphere. However, the deep subsurface is largely inaccessible at present, although such environments may need further consideration as technology advances and knowledge increases over time.

light of new science and technology. It is thus important to improve knowledge of organisms' ability to survive in various environmental conditions, as well as to improve bioburden reduction measures. A deeper understanding of psychrophiles and psychrotrophs may help in determining what is required to sterilize naturally occurring populations of these organisms and the extent of their ability to withstand mild or strong heat treatment. It would be especially valuable to know whether psychrophiles and psychrotrophs are sensitive to mild heat treatment that would not harm spacecraft components.

Recommendation 6. **NASA should sponsor research on those classes of microorganisms most likely to grow in potential martian environments. Given current knowledge of the Mars environment, it is most urgent to conduct research on psychrophiles and psychrotrophs, including their nutritional and growth characteristics, their susceptibility to freeze-thaw cycles, and their ability to replicate as a function of temperature, salt concentration, and other environmental factors relevant to potential spaceflight and to martian conditions.**

This recommended research should be expanded to include other classes of organisms if new scientific results suggest the existence of hydrothermal vents or other types of near-surface environments where microorganisms could grow, or as the ability to access deeper subsurface environments improves.

Over the past several decades, little research has been done to update the assigned embedded microbe density values used in NASA's planetary protection implementation requirements (Chapter 6). In the absence of new data, flight projects are required to assign values (number of microbes per cubic centimeter) for the bioburden implied by electronic piece parts or other nonmetallic materials used in spacecraft. Assigning these values amounts to reading an upper limit from a data table constructed on the basis of measurements made three decades ago. It is possible that modern materials, owing both to temperatures that some achieve during construction and to cleaner assembly environments, may have lower bioburdens than projects must currently assume. However, these details are not known. Research is needed on methods for determining actual levels of bioburden in encapsulated components for modern spacecraft materials, and models should be developed for extrapolating these levels to components that cannot be directly assayed.

Recommendation 7. **NASA should ensure that research is conducted and appropriate models developed to determine the embedded bioburden (the bioburden buried inside nonmetallic spacecraft material) in contemporary and future spacecraft materials. Requirements for assigned values of embedded bioburden should be updated as the results of such research become available.**

At present, dry-heat sterilization is the only method available for reduction of the microbial bioburden (as assessed through spore counts) of spacecraft hardware to Viking post-sterilization levels. Some newer materials and electronics, however, are incompatible with heat sterilization. Although NASA's implementation documents allow the use of alternative methods for reducing bioburden, NASA's requirements also stipulate that the user must provide conclusive data on biological effectiveness and on reproducibility, as well as demonstrate no reduction in hardware reliability (see Chapter 6). The potential for some techniques to leave organic residues is also important to examine when assessing alternative bioburden reduction techniques.

Recommendation 8. **NASA should sponsor studies of bioburden reduction techniques that are alternatives to dry-heat sterilization. These studies should assess the compatibility of these methods with modern spacecraft materials and the potential that such techniques could leave organic residue on the spacecraft. Studies of bioburden reduction methods should use naturally occurring microorganisms associated with spacecraft and spacecraft assembly areas in tests of the methods.**

Nonliving organic compounds, which may or may not have been originally derived from living organisms, could confound scientific investigations of Mars, especially those that involve the search for life. Consideration of nonliving organic contaminants should thus be an integral part of the requirements for spacecraft cleanliness. However, too little research has been conducted to understand the risks that nonliving contaminants, including possibly spacecraft propellants, might pose for life-detection or other scientific experiments.

Recommendation 9. **NASA should sponsor research on nonliving contaminants of spacecraft, including the possible role of propellants for future Mars missions (and the potential for contamination by propellant that could result from a spacecraft crash), and their potential to confound scientific investigations or the interpretation of scientific measurements, especially those that involve the search for life. These research efforts should also consider how propulsion systems for future missions could be designed to minimize such contamination.**

As discussed in Chapter 4, the potential for water distributed in the near surface and subsurface of Mars has significant implications with respect to preventing the forward contamination of Mars. Although recent missions are producing a wealth of data, scientific investigations have not yet yielded results detailed enough to distinguish among special and nonspecial regions on Mars. The committee believes that data from present and planned future missions also lack the fidelity to allow definitive conclusions about the distribution of water in the near subsurface; therefore, it finds that additional measurements are needed to understand with confidence the near-surface distribution of water on Mars and "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant Martian life forms" (COSPAR, 2003, p. 71)—that is, special regions. Examples of the kind of measurements needed, and the required spatial resolution and ground-truth that could make such a determination possible, are given in Chapter 4.

Recommendation 10. **NASA's Mars Exploration Office should assign high priority to defining and obtaining measurements needed to distinguish among special and nonspecial regions on Mars.**

TRANSITION TO A NEW APPROACH

Important to NASA's current planetary protection implementation requirements are detailed procedures for assessing and verifying the cleanliness of ATLO environments and spacecraft surfaces, as well as for documenting avoidance of recontamination before launch (see Chapter 2 and Appendix C). However, swab culturing is no longer the optimal way of determining ATLO environment or spacecraft bioburden. It can take up to 3 days to yield results—a period that adds to the time pressures of meeting spacecraft assembly deadlines and that makes enforcing planetary protection requirements more difficult.

Several methods are available that can more directly estimate the total viable cells on spacecraft and provide near-real-time results as compared with the spore-count assay methods currently used to assess bioburden on spacecraft (see Chapter 6). Nevertheless, NASA's progress in introducing alternative methods of bioburden assessment has been slow. The committee found no defined standard certification method or process in place for screening and approving promising new methods for assaying or reducing bioburden, nor any clear indication of how and when to discontinue the use of current methods based on culture growth. In addition, the absence of extensive comparative archival data about microbial diversity, as well as serious time and cost constraints, have limited the introduction of any innovative approaches to implementation of planetary protection policies.

The committee notes that the NRC's previous report on planetary protection for Mars urged that "efforts should be made to adopt current molecular analytical methods for use in bioburden assessment and inventory procedures for spacecraft assembly and launch for future missions, and also to develop new methods for the same purpose" (NRC, 1992, p. 18). Although some progress has been made along these lines (see Chapter 6), the committee believes that NASA should set a specific date for completion of a transition to use of advanced methods and should apply the necessary resources to meet that goal. The committee believes that, with a dedicated research program and appropriate budget, the transition to the new methods described here can be fully implemented in time for missions that will launch in 2016.

Recommendation 11. **NASA should take the following steps to transition toward a new approach to assessing the bioburden on spacecraft:**

- **Transition from the use of spore counts to the use of molecular assay methods that provide rapid estimates of total bioburden (e.g., via limulus amebocyte lysate (LAL) analysis) and estimates of viable**

bioburden (e.g., via adenosine triphosphate (ATP) analysis). These determinations should be combined with the use of phylogenetic techniques to obtain estimates of the number of microbes present with physiologies that might permit them to grow in martian environments.

- **Develop a standard certification process to transition the new bioassay and bioburden assessment and reduction techniques to standard methods.**
- **Complete the transition and fully employ molecular assay methods for missions to be launched in 2016 and beyond.**

The committee agrees with the 1992 NRC report that the probability of growth (P_g) of terrestrial microorganisms on Mars cannot currently be reliably estimated. However, the committee is concerned that, given current knowledge of Mars, P_g might in fact prove to be greater than suggested by earlier reports (NRC, 1978, 1992). The committee considered returning to a P_g-based approach to planetary protection but concluded that the unknowns remain too great to do so now (see Chapter 5).

However, implementation of the DNA phylogenetic methods recommended in this report, coupled with increasing knowledge of the martian environment, might make it possible to return to a P_g-based approach with much greater confidence. Once spacecraft bioburden is understood on a species-by-species basis, it may prove possible to radically alter current planetary protection requirements and protocols. For example, it may be proved that broad classes of organisms have essentially no chance of growth at a particular martian landing site, whereas, say, psychrophiles may have nonvanishing probabilities of growth. In this case, Equation 5.1 could be employed to demonstrate that no particular bioburden reduction need be employed for most organisms on the spacecraft. However, certain species, were they present, would have to be rigorously eliminated. In some cases, elimination of particular species on spacecraft components might prove easier and less demanding than with Viking-style baking. Greater knowledge of the diversity and number of species on outbound Mars spacecraft is critical. Such data are important in reducing uncertainty in assessments of the bioburden reduction needed to prevent the forward contamination of Mars.

INTERIM REQUIREMENTS

The recommendations in this report are intended to provide a path toward a transition by NASA to planetary protection practices and policies that will reflect current science and technology. A time line for the implementation of these recommendations is offered in Chapter 9. The committee anticipates that it will take until 2016 before R&D efforts can be conducted and their results used to develop updated planetary protection methods and techniques that are fully implemented on missions to Mars. However, because a number of Mars missions are planned for launch prior to 2016, the committee recognizes the need for an interim plan that updates existing planetary protection requirements to reflect new scientific knowledge about Mars and terrestrial microorganisms.

The recommendations presented below, based on existing planetary protection protocols, concern planetary protection requirements that should be implemented during the interim period from now until the transition to new practices is completed. These recommended requirements are intended to reflect the best current scientific understanding of terrestrial microorganisms and the martian environment, incorporating the new knowledge acquired since publication of the 1992 NRC report. Because knowledge of Mars is changing so rapidly, the committee has tried to build appropriate flexibility into these requirements.

Recommended Changes for Category IV Missions

Chapter 2 of this report describes the current COSPAR planetary protection categories, based on the destination body in the solar system and type of mission to be flown (see Table 2.2). Category III applies to flyby and orbiter missions that should not make direct contact with a planet or its atmosphere. Category IV applies to direct-contact missions, including landers, penetrators, and atmospheric probes.

Determining the level of bioburden reduction needed to avoid confounding a mission's own measurements (for life detection or otherwise) is scientifically crucial, and it is the responsibility of a given mission's planners.

Failure to reduce bioburden or certain nonliving contaminants appropriately, and thus to prevent payload investigations from being compromised, would be a mistake that could cause partial mission failure. Different levels of cleanliness, both with respect to viable microorganisms (Chapter 6) and with respect to nonliving contaminants (Chapter 7), might be required for missions flying different kinds of instruments, and so there is no single or uniform requirement that planetary protection should impose for this purpose. In particular, some types of biodetection experiments may require extraordinary levels of cleanliness, whereas others may have much less stringent requirements.

Thus the committee believes that the IVa and IVb mission categories are no longer a useful way to determine planetary protection requirements for Mars landers. Rather, Mars mission categories should depend on spacecraft destination, regardless of mission instrument payload. To avoid confusion with Categories IVa through IVc, the committee defines new categories—IVs and IVn. Category IVs applies to missions that are landing or crashing in, or traversing, excavating, or drilling into, special regions. In contrast, Category IVn missions are those that are not going to a special region.

Recommendation 12. **For the interim period until updated planetary protection methods and techniques can be fully implemented,**

- **NASA should replace categories IVa through IVc for Mars exploration with two categories, IVn and IVs. Category IVs applies to missions that are landing or crashing in, or traversing, excavating, or drilling into, special regions; Category IVn applies to all other category IV missions.**
- **Each mission project should (in addition to meeting the requirements imposed by Categories IVn and IVs) ensure that its cleanliness with respect to bioburden and nonliving contaminants of concern is sufficient to avoid compromising its experiments, in consultation with NASA's planetary protection officer.**

As discussed in Chapter 4, scientific results from recent Mars orbiters and landers have confirmed the existence of past water on Mars and suggest that liquid water may currently exist, at least transiently, on the planet. Recent results make it substantially more likely that transient liquid water may exist near the surface at many locations on Mars, and it is difficult on the basis of current knowledge to declare with confidence that any particular Mars regions are free of this possibility. Researchers do not currently have the data necessary to distinguish special regions on Mars from regions that are not special. Current and planned investigations of water on Mars, although very important, involve issues of spatial resolution and interpretive ambiguities that render them unlikely to fully resolve this ambiguity. Along with these ambiguities are uncertainties regarding the diversity of the viable bioburden on spacecraft that could make contact with the martian surface, as well as the potential for such organisms to grow in martian environments. The committee thus found that information is currently insufficient to make use of Category IVn. Until measurements are made that permit distinguishing confidently between regions that are special on Mars and those that are not, NASA should treat all direct-contact missions (i.e., all Category IV missions) as being in Category IVs.

Recommendation 13. **Until measurements are made that permit distinguishing confidently between regions that are special on Mars and those that are not, NASA should treat all direct-contact missions (i.e., all Category IV missions) as Category IVs missions.**

The independent panel referred to in Recommendation 4 could be the body that recommends, as more knowledge becomes available, whether areas may be appropriately designated as Category IVn rather than Category IVs.

Note added in proof—The following text change was approved and made after release of the prepublication copy of this report: The phrase "in consultation with NASA's planetary protection officer" was added to Recommendation 12.

Bioburden Reduction Requirements for Category IV Missions

The committee considered at length a range of levels of bioburden reduction appropriate to Category III and Category IVn and Category IVs missions. It evaluated a number of factors:

- *Definition of "special region."* A "special region" (see Chapter 2) is defined as "a region within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms. Given current understanding, this is to apply to regions where liquid water is present or may occur" (COSPAR, 2003, p. 71). The COSPAR IVc classification (see Chapter 2) was needed in part to prevent a "dirty" spacecraft (one sterilized to only IVa, or Viking pre-sterilization, levels; see Table 2.1) from being sent to a special region, even if that particular spacecraft did not have life-detection experiments on board. Therefore, the committee could not see permitting a Category IVs mission to fly with only Viking pre-sterilization bioburden protection. Moreover, microorganisms could be introduced into a potential liquid water environment not only by sampling arms or rover wheels that might make direct contact with Mars, but also from any exposed surface on the spacecraft from which a microorganism might be saltated[6] or lofted into contact with that environment.
- *Kinetics of growth and the period of biological exploration.* At the same time, the committee concluded that, on the basis of current understanding, Mars surface or near-surface environments are likely to be cold ones where liquid water, if ever present, was present only diurnally (and perhaps even then only seasonally) (see Chapter 4). It might be possible for certain Earth microorganisms to survive under such conditions, but extrapolations from Earth experience suggests that most terrestrial organisms would not survive, and that those that would survive (most likely, psychrophilic or psychrotrophic organisms) would likely have slow growth kinetics, that is, long generation times—long enough so that during the period of biological exploration, the organisms would not produce sufficient copies of themselves to pose a likely threat to life-detection measurements made during that period (see Chapter 5).
- *Possibility of long-lived water.* There could be some surface or near-surface locations on Mars, as well as deeper subsurface locations, where long-lived liquid water could be present (see Chapter 4). None has yet been unambiguously observed, although such environments, at least in the subsurface, are widely anticipated. A dramatic example would be the discovery of hydrothermal vents at the martian surface. Sites of long-lived liquid water could be particularly amenable to the sustained growth of certain Earth microorganisms.
- *Probability of a crash.* Table 1.1 displays the outcome of all missions of all nationalities sent from Earth to Mars, and in particular, tracks which of these missions failed, and of those that failed, which crashed onto the martian surface. If the two penetrators associated with the Mars Polar Lander mission are counted as having failed after landing, the compilation shows that 12.5 percent of U.S. Mars landers have crashed. Historically, one in eight U.S. Category IV missions has crashed. Crashes may take many different forms and have varied consequences, however. For example, supersonic impacts would likely expose all surviving surfaces to the martian environment, and even some embedded bioburden. However, many, but not all, spacecraft components would in such a case experience great bioburden reduction through extreme surface heating. The most likely type of crash impact, terminal impact, would expose only some of the nominally nonexposed surfaces. Some missions, such as those using martian airplanes, might involve low-velocity crashes as part of a nominal mission plan. Penetrator missions are designed for high-speed impact, but they can also break up if their angle of attack is too great at impact. Under certain crash scenarios, higher levels of planetary protection would be required. However, making this determination requires detailed knowledge of each particular mission, including the landing technique and its history of success or failure, the nature of the lander, the presence or absence of radioisotope thermal generators (discussed below), the nature of the landing site, and many other factors. Therefore, a mission-by-mission analysis will be necessary.[7]

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[6]"Saltation" is the process by which a small particle is lifted off a surface without enough velocity to place it into suspension, but with enough velocity to move it downwind in a series of little jumps.

[7]The committee was briefed on the details of one such mission analysis by Brian K. Muirhead, chief engineer, JPL, "Mars Science Laboratory Planetary Protection Categorization Strategy," briefing to NRC Space Studies Board, May 5, 2004, Diversa Corporation, San Diego, California.

TABLE 8.1 Recommended Levels of Bioburden Reduction for the Interim Period

Level	Requirement	Representative Scenario
1	Viking lander pre-sterilization total bioburden (fewer than 3×10^5 total surface spores) and 300 spores per square meter.[a]	Category IVn
2	Viking pre-sterilization levels required for the bulk spacecraft plus Viking post-sterilization on all exposed surfaces.[a,b] The latter is to be understood as an areal (surface density) measurement. Explicitly, Viking post-sterilization levels correspond to a reduction of 1×10^{-4} times the Viking pre-sterilization upper limit of 300 spores per square meter.	All Category IVs
3	Viking pre-sterilization levels required for the bulk spacecraft [b] plus Viking post-sterilization on all surfaces, including those not exposed under nominal (e.g., no-crash) conditions.[a] Explicitly, Viking post-sterilization levels correspond to a reduction of 1×10^{-4} times the Viking pre-sterilization upper limit of 300 spores per square meter.	Category III missions that do not meet existing requirements for probable orbital lifetime
4	Viking post-sterilization bioburden reduction for the whole spacecraft.[a] Currently, this would likely mean baking the spacecraft in a manner similar to that employed in the Viking mission, although the committee encourages NASA to investigate other technologies to this same end.	Category IVs missions accessing locations determined to have long-lived liquid water
5	The committee cannot currently specify the technology that could become available to attain zero microorganisms on Mars-bound spacecraft. Bioburden reduction techniques more effective than those applied today may be or may soon be available for use on spacecraft (see Chapter 6). A level 5 bioburden reduction level would represent the implementation of these techniques, to achieve bioburden reduction significantly more rigorous than that obtained for the Viking landers.	Category IVs missions accessing locations determined to have long-lived liquid water

[a]See Table 2.2 for descriptions of Viking pre- and post-sterilization requirements.
[b]"Bulk spacecraft" refers to the entire nonmetallic volume of the spacecraft, including entry, descent, and landing systems. For missions that include both orbiters and direct contact components, the appropriate level must be determined separately for each type of component. An exposed surface is a surface that freely communicates with the martian atmosphere or surface. All external surfaces on a lander would count as exposed surfaces, but interior surfaces might do so as well if they were not fully enclosed or shielded from the atmosphere by submicron filters.

• *Potential for radioisotope thermal generators to create liquid water.* Some future landers may include radioisotope thermal generators (RTGs) or possibly even nuclear reactors. Under some crash conditions, RTGs could be released from the spacecraft yet remain intact or largely intact. In such a scenario, it might be possible, given certain crash site characteristics, for an RTG to produce sufficient heat to create its own long-lived liquid water environment. That result is far from guaranteed, owing both to the many different crash outcomes that are possible and to the thermal output of the particular RTG to be flown (e.g., if heat is conducted away sufficiently quickly, melting may never take place).

The committee considered all these factors,[8] with due regard for the substantial uncertainties that they present, with respect to Mars, Earth microbiology, and particular characteristics of future missions and corresponding crash scenarios. Consequently, the committee recommends a new set of levels for bioburden reduction for Category IV missions. The interim classification scheme presented below is based on current protocols (i.e., Viking-era protocols and the use of spore counts for determining bioburden) that should be put in place until the committee's recommendations for the transition to modern methods are implemented.

[8]This included consideration of input from Brian K. Muirhead, chief engineer, JPL, "Mars Science Laboratory Planetary Protection Categorization Strategy," briefing to NRC Space Studies Board, May 5, 2004, Diversa Corporation, San Diego, California.

The committee concluded that five levels of bioburden reduction would be sufficient, during the interim period envisioned, to cover appropriate bioload requirements for Category III and IV missions to Mars (see Table 8.1).

The committee considered the level of bioburden reduction that should apply to different categories of missions, taking particular account of the findings described in Chapters 4 and 5. The committee found that Category IVn missions should satisfy level 1 bioburden requirements. Until NASA obtains further measurements to distinguish special regions on Mars from nonspecial regions, Category IVn will be a null set. Therefore, all Category IV missions for the time being should be treated as Category IVs missions.

Recommendation 14. **NASA should ensure that all Category IVs missions to Mars satisfy at least level 2 bioburden reduction requirements.[9] For each Category IVs mission, NASA's planetary protection officer should appoint an independent, external committee with appropriate engineering, martian geological, and biological expertise to recommend to NASA's planetary protection officer whether a higher level of bioburden reduction is required. This analysis should be completed by the end of Phase A (performance of the concept study) for each mission.**

Key points that the appointed committee should consider in reaching this determination include previous spacecraft experience, crash scenarios, and modeling for the mission, including the likely extent of release to the martian environment of organisms on nominally nonexposed surfaces or embedded in spacecraft components, as well as the presence and likely fates of radioisotope thermal generators or other sources of significant heat present on the spacecraft.

Recommendation 15. **NASA should sponsor research on how to implement level 3, 4, and 5 bioburden reduction requirements in practical ways. This research should include techniques to reduce the surface bioburden to post-Viking sterilization levels, and maintain those levels, for both exposed and nonexposed spacecraft surfaces, through new techniques (such as the use of hydrogen-peroxide vapor), component bagging, heating, or other means.**

Recommendation 16. **Any mission to Mars that will access regions or sites that have been determined to have or are strongly suspected to have long-lived liquid water should satisfy at least level 4 bioburden reduction requirements.**

Category III Requirements

Category III missions, typically orbiters, are missions that are not expected to be direct-contact missions (see Chapter 2) but that have the potential for crashing on Mars (see Table 1.1 for the crash history of such missions). Category III mission crashes will be supersonic crashes, following extreme heating of many, but not all, spacecraft components.

Note added in proof—The following text changes were approved and made after release of the prepublication copy of this report: In Recommendation 14, a footnote was added to the first sentence; in the second sentence, the word "determine" was replaced by the phrase "recommend to NASA's planetary protection officer."

[9]In this chapter, the committee defines level 2 as corresponding to the Viking-level pre-sterilization required for the bulk spacecraft plus Viking post-sterilization for all exposed surfaces; the latter is to be understood as an areal (surface density) measurement. Explicitly, Viking post-sterilization levels correspond to a reduction of 1×10^{-4} times the Viking pre-sterilization upper limit of 300 spores per square meter. Level 2 requirements (see Table 8.1) are not identical to those previously applied to Category IVs missions (Table 2.2), as is readily seen by comparing Tables 8.1 and 2.1. The committee also draws a distinction between mission categorization (based on mission destination) and bioburden reduction levels; e.g., Category IVs missions will typically be level 2 missions, but under some circumstances a decision could be made to require level 3 or higher for a particular Category IVs mission.

Recommendation 17. **NASA should take the following approach to preventing the forward contamination of Mars from Category III missions:**

1. Category III missions should be required to have orbital lifetimes of 20 years and 50 years, and the probability of impact over those time periods should be below 1 percent and 5 percent, respectively.
2. Category III missions unable to meet these requirements should satisfy at least level 3 bioburden reduction requirements.
3. For each Category III mission that cannot meet the orbital lifetime requirements, NASA's planetary protection officer should appoint an independent, external committee with appropriate engineering, martian geological, and biological expertise to recommend to NASA's planetary protection officer whether a higher level of bioburden reduction is required. This analysis should be completed by the end of Phase A for each mission. In reaching this determination, the appointed committee should consider previous experience, crash scenarios, and modeling for the mission, including the likely extent of release to the martian environment of organisms on nominally nonexposed surfaces or embedded in spacecraft components, as well as the presence and likely fates of radioisotope thermal generators or other sources of significant heat present on the spacecraft.

Chapter 9 presents a roadmap showing how the various recommendations fit together, as well as a time line for meeting the milestones that are required.

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9

Transition Process and Time Line

As stated in Chapter 8, the committee concluded that rapidly expanding knowledge of Mars, significant technology advances in microbiology, and a growing awareness of the expanding limits of Earth life together require a transition in NASA's approach to preventing the forward contamination of Mars. This chapter outlines an approach and schedule for this transition.

APPROACH

Achieving a comprehensive revision of Mars planetary protection policy and protocols pertaining to forward contamination of Mars depends on meeting four objectives: (1) assessment of spacecraft contaminants, (2) definition and development of revised requirements for reduction of bioburden, (3) improvement of bioburden reduction techniques, and (4) validation of and transition to new standards and techniques. In Chapter 8 the committee also recommends planetary protection categories and bioburden reduction requirements that NASA should implement as interim requirements (recommendations 12 to 17). Those interim requirements represent updates to bioburden reduction measures reflecting recent scientific understanding about Mars and microbiology; they should be applied concurrently until these four objectives are fully implemented. The relationships among the current approach to planetary protection, programmatic support, needed research and reconnaissance, interim requirements, and transition to a new approach are summarized in Figure 9.1

Each of the four objectives to be met in completing a revision of current Mars forward contamination protocols is described below.

Objective 1: Assessment of Spacecraft Contaminants

The purpose of assessing spacecraft contaminants is to determine which microbes present in the construction, testing, and launch of Mars missions actually threaten either to compromise Mars science or to contaminate the Mars environment. Such an assessment requires (1) assaying to determine the exact nature of the bioburden acquired in flight system development, assembly, and test facilities and (2) determining what fraction of this bioburden could actually threaten to contaminate the Mars environment or confound planned life-detection measurements. Identifying the microbial content of the bioburden—including both the surficial and the embedded

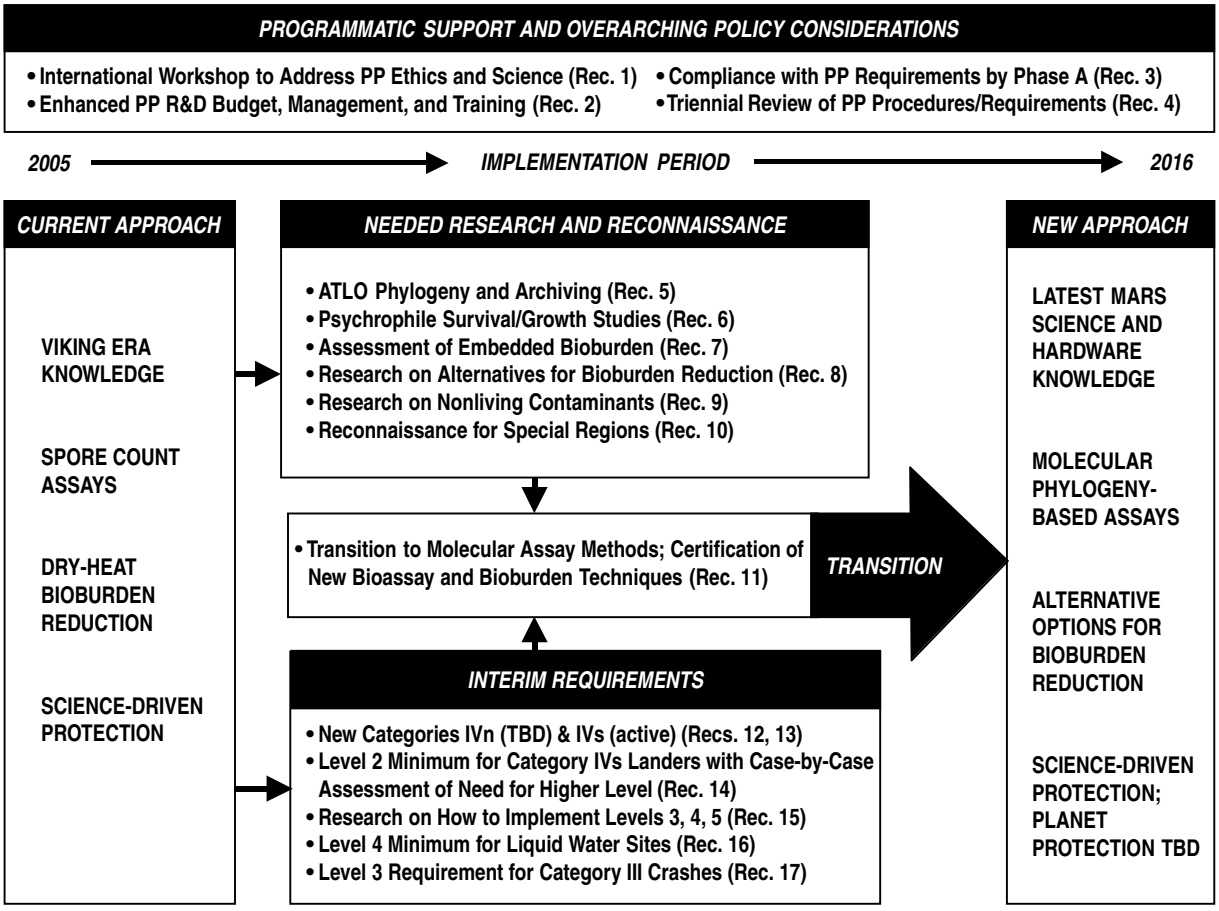


FIGURE 9.1 Proposed framework for a transition from the current approach to a new approach to Mars planetary protection (PP), along with the programmatic support and global policy considerations required to make the transition.

bioburden of all materials in Mars flight systems, as well as in the facilities within which the systems are assembled, tested, and ultimately launched—is necessary to assess which species are capable of surviving interplanetary transit and then growing in the Mars environment. Knowing which species are dead, or will die en route to Mars, is equally important. (Committee recommendations 1, 2, 5, 6, 7, and 10 pertain to objective 1.)

Objective 2: Definition and Development of Revised Requirements for Reduction of Bioburden

Characterization of the bioburden on spacecraft (objective 1) and the ability to target those microbial populations of greatest concern for contaminating Mars enable objective 2, review and revision of existing bioburden reduction standards in NASA's requirements documents. Revised requirements will set limits on microbes based on understanding of the likelihood that these populations will either contaminate the Mars environment through growth or will confound life-detection measurements by their mere existence (dead or alive) on the spacecraft or in contact with the Mars environment. (Committee recommendations 1, 2, 8, 10, and 11 pertain to objective 2.)

Objective 3: Improvement of Bioburden Reduction Techniques

Alternatives are needed for bioburden reduction methods that more effectively reduce or eliminate species-specific bioburdens, or that place less stress on spacecraft and instrument components. Knowing what bioburden must be reduced and where is necessary to determining when and how bioburden reduction can be accomplished and maintained throughout all the physical systems associated with developing the flight system. However, to evaluate the effectiveness of objective 3, the process of improving bioburden reduction methods and testing alternatives can begin simultaneously with pursuit of objective 1, thus possibly reducing the time for transitioning to new methods. Perhaps the most likely microbial threats to Mars can be eliminated with bioburden reduction processes that are less stringent and possibly less costly than current practices—for example, processes such as lower-temperature heat sterilization. Knowledge of materials' ability to tolerate alternative sterilization techniques was critical during development of the Viking heat sterilization process and will be similarly important in ongoing research efforts, given the availability of new spacecraft materials (plastics, composites, metals, etc.), as will additional information on exactly what is to be sterilized. (Committee recommendations 1, 2, 5, 6, 7, 8, 10, 11, and 15 pertain to objective 3.)

Objective 4: Validation of and Transition to New Standards and Techniques

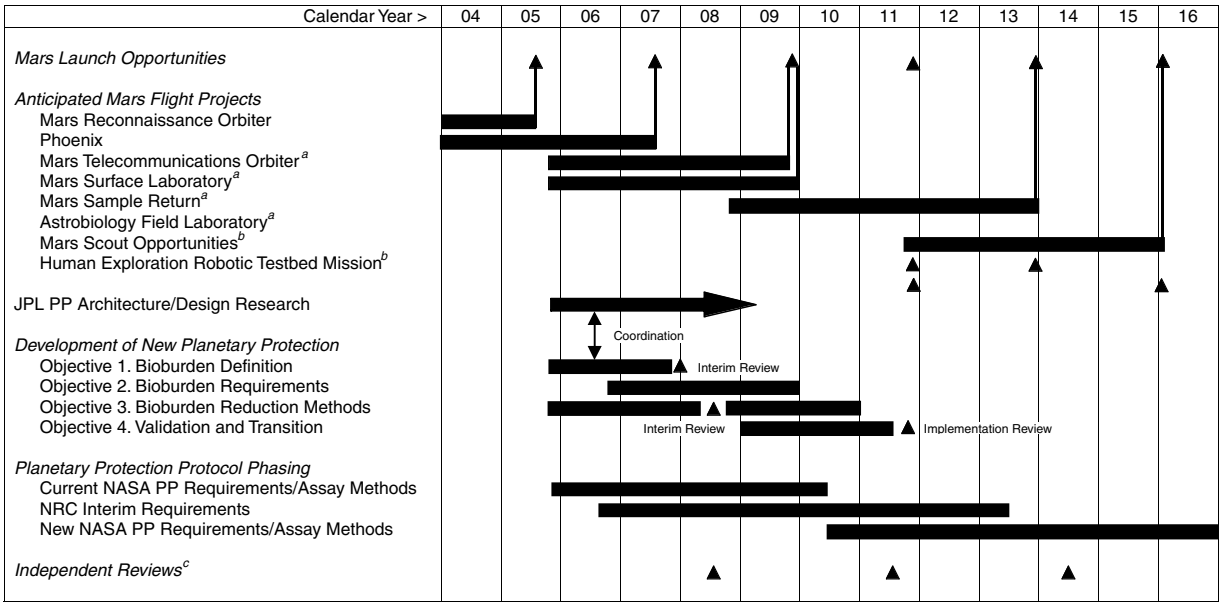
Changes to policy and procedures for planetary protection proposed in response to the committee's recommendations must be validated during a period of demonstration and testing before existing bioburden reduction requirements are replaced. Validation would also involve presentation of new requirements to COSPAR for acceptance. Once validated and certified, new approaches could then be applied with the confidence that they would work as expected, and old approaches could be phased out. Data obtained during this period of comparison could also be used to reassess the potential contamination that may already exist on Mars as a result of previous missions. A validation period could easily involve several Mars missions spanning several launch opportunities (i.e., more than 5 years), although the committee recommends that the implementation be fully operational by 2016. (Committee recommendations 1 through 11 and 15 pertain to objective 4.)

IMPLEMENTATION TIME LINE

Given the rapid advances in in situ science instrument capabilities and the possibility of contamination in a Mars environment potentially richer in water than previously believed, it is important to review and adjust Mars forward contamination requirements and procedures expeditiously. That said, the earliest chance to alter Mars planetary protection procedures and begin to demonstrate, verify, and validate new methods from the ground up would likely be on the next new (not yet in development) flight project, that is, the 2011 Mars Scout mission. Development of the next program-directed mission, possibly a Mars Sample Return mission to be flown in 2013, will probably also begin at the same time as development of the 2011 Mars Scout mission, because the sample return mission is expected to be more complex and to require more development time before launch. Hence, there will be an opportunity during FY 2008, when development of both the 2011 and 2013 Mars missions is expected to begin, to test and demonstrate the effectiveness of new bioburden reduction requirements and procedures being researched and developed in conjunction with objectives 1 and 3. A new, completely validated set of protocols for planetary protection employing advanced bioassay and bioburden reduction methods would more realistically be implemented on a mission developed for launch early in 2016. A transition to use of such protocols would have to be initiated no later than the beginning of FY 2012.

A proposed schedule based on these considerations, along with the development periods for all current and planned missions through the 2016 launch, is depicted in Figure 9.2.[1] Note that the planned JPL Planetary

[1]A FY 2006 start date of the committee's proposed time line could depend on NASA's ability to access or reprogram resources to devote to the recommended research efforts.



[a]Flight projects in concept definition stage subject to changes in definition and launch date.
[b]Mars program elements with repetitive flight projects (opportunities shown are placeholders).
[c]Triennial reviews of Mars knowledge base, program plans, and planetary protection requirements (recommendation 4).

FIGURE 9.2 Proposed schedule for the revision of Mars planetary protection requirements. PP, planetary protection. NOTE: Launch schedule for future missions is based on information provided in Mars Science Program Synthesis Group, *Mars Exploration Strategy 2009-2020.* Jet Propulsion Laboratory, JPL 400-1131, 2004.

Protection Architecture/Design Research study represented in the center of Figure 9.2 shares several of the objectives of the approach outlined here.[2] Coordination of the plan and schedule proposed in Figure 9.2 with the planned JPL effort is clearly warranted. At NASA's discretion, this existing work might even be integrated with the approach and schedule suggested in this report.

Because the results of each objective discussed above feed into and affect subsequent objectives, periodic review of research progress by an independent panel is advisable (Recommendation 4). The results of research on characterizing bioburden, defining requirements, and developing techniques for reduction of bioburden must be verified, validated, and certified before new methods can be adopted as standard techniques. The committee recognizes, as a separate matter, the importance of maintaining a dialogue and interface with COSPAR and other entities to ensure concurrence on a process that would clearly change how NASA complies with globally acceptable planetary protection protocol.

This approach to modernizing Mars planetary protection clearly illustrates that changing NASA's approach so that it embraces advances in microbiology along with current understanding of Mars cannot be done quickly. Even an aggressive plan such as the one outlined here will take the better part of a decade to complete and fully apply to the Mars Exploration Program.[3] There is thus every reason to begin the pertinent work as quickly as possible.

[2]JPL has proposed an architecture study that is considering planetary protection requirements in the architecture, design, manufacturing, assembly, and testing of spacecraft. The study will also consider the vulnerability of spacecraft (components) to Viking-level heat sterilization and how to address this and other planetary protection factors at a system architecture level.

[3]This is one reason that the committee has advised measures (recommendations 12 to 17) for implemention in the interim.

Appendixes

A

Biographical Sketches of Committee Members and Staff

CHRISTOPHER F. CHYBA, *Chair,* holds the Carl Sagan Chair for the Study of Life in the Universe at the SETI Institute and is associate professor in the Department of Geological and Environmental Sciences and co-director of the Center for International Security and Cooperation at Stanford University. Dr. Chyba is a MacArthur Fellow for his work in astrobiology and international security and a former member of the National Security Council staff. His scientific research focuses on planetary habitability and the search for life elsewhere in the solar system. He is a past member of the NASA Astrobiology Institute's Executive Council, former chair of the Science Definition Team for NASA's Europa Orbiter Mission, and a former member of NASA's Space Science Advisory Committee's Executive Committee, among numerous other service positions. Dr. Chyba currently serves on the National Academy of Sciences' Committee on International Security and Arms Control and on the National Research Council's (NRC's) Committee on Advances in Technology and the Prevention of Their Application to Next Generation Biowarfare Threats. Since July 2005, Dr. Chyba has been a professor of astrophysical sciences and international affairs at Princeton University, joint in the Woodrow Wilson School for Public and International Affairs and the Department of Astrophysical Sciences.

STEPHEN CLIFFORD is a staff scientist at the Lunar and Planetary Institute. His research focuses on geology, subsurface hydrology, meteorology, glaciology, and soil physics to characterize the dynamics of climate and water on Mars. In particular, his research has examined evidence concerning the martian hydrosphere and how it may have evolved over time. Dr. Clifford is also a principal investigator in NASA's Planetary and Geophysics Program and has served on several NASA review panels. He received his M.S. and Ph.D. in astronomy from the University of Massachusetts, Amherst.

ALAN DELAMERE is senior engineer and program manager at Ball Aerospace and Technology Corporation. He is currently involved as co-investigator on the Mars Reconnaissance Orbiter (MRO) High Resolution Imaging Science Instrument (HIRISE) and on the Deep Impact mission to Comet Tempel 1. Mr. Delamere has been involved in the Mars program since the 1980s. His expertise focuses on instrument building and mission design.

MARTIN S. FAVERO is the director of Scientific and Clinical Affairs, Advanced Sterilization Products, a Johnson & Johnson Company. Previously, he served in several positions at the Centers for Disease Control (CDC) and Prevention, including director of CDC's Hospital Infections Program and deputy director of the Hepatitis

Laboratories Division and chief of its Applied and Environmental Microbiology Section, and he headed the CDC's work in spacecraft sterilization and planetary quarantine from 1964 to 1972. Dr. Favero has published more than 200 papers and book chapters on public health, environmental microbiology, disinfection and sterilization, environmental aspects of viral hepatitis, dialysis-associated disease, biosafety, and prevention of viral hepatitis and AIDS transmission in health care settings. He is a member of Sigma Xi, a fellow of the Infections Diseases Society of America, and a fellow of the American Academy of Microbiology. He has served in numerous capacities for professional societies, including as the president of the American Society for Microbiology, Arizona Branch. Dr. Favero served on the NRC Committee on Joint Medical/Engineering Approaches for Reducing Nosocomial Infection (1997 to 1998).

ERIC J. MATHUR is vice president of scientific affairs and molecular diversity at Diversa Corporation. His scientific responsibilities include managing Diversa's Biodiversity Access Program, involving relationships with more than 15 countries and institutions. He also has oversight of and directs Diversa's molecular diversity program, which involves the construction of environmental gene libraries and the development of directed evolution and pathway engineering technology at Diversa. Mr. Mathur is a member of Diversa's Senior Management Committee and its Internal Product Review Committee and has served on both Sygenta and Dow Chemical research advisory committees. He has published more than 40 papers, is named inventor on more than 25 U.S. patents, and has been invited to present more than 100 lectures. Mr. Mathur also sits on the Scientific Advisory Board of the Thermal Biology Institute, is a member of the SETI Institute's Life in the Universe working group, and holds a visiting scientist position at the International Center for Insect Physiology and Ecology in Kenya. Additionally, Mr. Mathur serves as an editor of *Extremophiles*, and he is on the Scientific Advisory Committee of the Department of Energy's Joint Genome Institute.

JOHN C. NIEHOFF is senior research engineer and corporate vice president of Science Applications International Corporation (SAIC), where he is active in program planning and assessment support primarily to the Space Science Enterprise at NASA. He also serves as senior staff member of the Space, Earth, and Atmospheric Sciences Group within SAIC. Previously, he managed SAIC Space Science Operations, which included six divisions focusing on NASA basic research, program planning and evaluation, and data analysis. Mr. Niehoff is the 1981 recipient of the NASA Public Service Award and more recently the recipient of several NASA/Langley Research Center Team excellence awards (Discovery and Pluto Kuiper Belt Announcement of Opportunity evaluations).

GIAN GABRIELE ORI is a professor of geology (Faculty of Science, Universita G. d'Annunzio, Italy) and director of the International Research School of Planetary Sciences (Pescara, Italy). He also holds a visiting professor position at the Institut d'Estudis Espacials de Catalunya, Universitat Politecnica de Catalunya, Barcelona, Spain. Professor Ori is an interdisciplinary scientist for geology and is co-investigator of the high-resolution stereo camera and co-investigator of the subsurface penetrating radar MARSIS on the Mars Express mission. He is also associate to the synthetic aperture radar for the study of Titan's surface on the Cassini mission and to the team of SHARAD of the subsurface radar sounder on the Mars Reconnaissance Orbiter. Professor Ori has been leader of the Surface Analysis Group of the Exobiology Science Team of the European Space Agency and of the Preliminary Science Definition team of the Mars Sample Return ESA mission.

DAVID A. PAIGE is an associate professor of planetary science at the University of California, Los Angeles. His research interests include studies of the surface, atmosphere, polar caps, and climate of Mars; the polar caps of Mercury, the Moon, Triton, and Pluto; Earth's climate; and remote sensing and spacecraft exploration. He was the principal investigator for the Mars Volatiles and Climate Surveyor instrument package on the Mars Polar Lander spacecraft. Dr. Paige served on the NRC Committee on Planetary and Lunar Exploration (1998 to 2001) and on the Committee on Assessment of Mars Science and Mission Priorities (2001).

ANN PEARSON is an assistant professor of biogeochemistry in the Department of Earth and Planetary Sciences at Harvard University. Her research interests cover light isotope biogeochemistry, including applications of com-

pound-specific isotopic analysis to early diagenesis of sedimentary organic matter; anthropogenic perturbation of organic reservoirs and terrestrial carbon dynamics; bacterial pathways of carbon assimilation; environmental ecology and metabolism of Bacteria and Archaea; new interfaces for mass spectrometry with applications to environmental samples; and phylogenetically directed research on problems in organic geochemistry and environmental microbiology. Dr. Pearson has developed entirely new analytical techniques that link genomics, biochemistry, and radio-carbon dating in the context of geochemistry. Also, she is using these new methods in work toward redefining how life emerged and evolved on this planet in the context of the geosystem. She is a member of the American Geophysical Union (AGU) and the American Chemical Society and served as a member of the 2003 National Science Foundation (NSF) Review Panel for Molecular and Cellular Biosciences, Microbial Observatories, and Microbial Interactions and Processes (MO/MIP); the 2003 Harvard University Curricular Review: Working Group on General Education; and the 2002-2003 Planning Committee for Harvard Microbial Sciences Initiative.

JOHN C. PRISCU is a professor of ecology in the Department of Land Resources and Environmental Sciences at Montana State University. His research focuses on nutrient biogeochemistry, microbial ecology, life in extreme environments, and astrobiology. He is currently conducting research on decontamination techniques for sample collection and return from Lake Vostok, and on the novel genomes and physiologies in the lakes of the McMurdo Dry Valleys. Also, he is a principal investigator on the McMurdo Dry Valley Long-Term Ecological Research project, which uses the dry valleys as monitors for global change. Dr. Priscu has led research teams to Antarctica for 20 years, and he convenes an international group of specialists to develop a plan to sample Antarctica subglacial lakes. He received the Goldthwait Award in 2003 from the Byrd Polar Research Center for his studies of microbial life in polar ice. He was a member of the AGU Antarctic Research Series Editorial Board for a number of years, is a member of the U.S. Ice Core Working Group, serves on the Advisory Committee for the U.S. Ice Core Drilling Service, and is currently the U.S. representative for Life Science on the Scientific Committee on Antarctic Research. He served on the NRC Committee on Frontiers in Polar Biology.

MARGARET S. RACE is a scientist with the SETI Institute whose research focuses on planetary protection and analysis of cross-contamination both in space and on Earth. Her current studies involve legal and regulatory aspects of Mars sample return proposals; involvement of the public in the review and approval process for sample return; ethical implications of the possible discovery of life beyond Earth; and educational outreach about astrobiology through schools, museums, and the mass media. Previously, Dr. Race served on the NRC Task Group on Issues in Mars Sample Return (1996 to 1997), Study on Transportation in a Sustainable Environment (1994 to 1997), Task Group on Sample Return from Small Solar System Bodies (1997 to 1998), and Committee on Development of an Addendum to the National Science Education Standards on Science and Technology (1998 to 2001).

MITCHELL L. SOGIN is director of the Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory. His research interests emphasize molecular phylogeny and the evolution of eukaryotic ribosomal RNAs. He is a member of the American Society of Microbiology, the Society of Protozoologists, the International Society of Evolutionary Protozoologists, the Society for Molecular Biology and Evolution, the American Association for the Advancement of Science, and the American Society for Cell Biology. Dr. Sogin is a former member of the Space Studies Board (1999 to 2004), an associate fellow of the Canadian Institute for Advanced Research, a division lecturer for the American Society of Microbiology, a recipient of the Stoll Stunkard Award from the American Society of Parasitologists, a fellow of the American Academy of Microbiology, and a visiting Miller Research Professor at the University of California at Berkeley.

CRISTINA TAKACS-VESBACH is an assistant professor in the Department of Biology at the University of New Mexico at Albuquerque. Dr. Takacs-Vesbach conducted her graduate work in microbial ecology and performed research on the factors affecting bacterioplankton distribution and productivity in the dry valley lakes of Antarctica. Her postdoctoral work focused on the microbial phylogenetic and physiological diversity of hydrothermal springs.

Dr. Takacs-Vesbach is the recipient of the outstanding doctoral student award of the Montana State University (MSU) Foundation, the John Wright Award for Limnology, and the Gary Lynch Award from the MSU Department of Biology.

Staff

PAMELA L. WHITNEY, study director, is a senior program officer at the Space Studies Board, where she has directed studies and workshops on international cooperation in space, Earth remote sensing, Mars planetary protection, and space policy, among other space technology and research topics. Ms. Whitney also serves as the executive secretary of the U.S. national committee to the Committee on Space Research (COSPAR) of the International Council for Science (ICSU). Previously, she held positions as an analyst at the aerospace consulting firm CSP Associates, Inc., and as a researcher and writer for Time-Life Books, Inc. Ms. Whitney was president of Freelance Unlimited and held contracts with the National Geographic Society, the World Bank, and the U.S. Congress Office of Technology Assessment. Ms. Whitney holds an A.B. in economics from Smith College and an M.A. in international communication from the American University. She is a member of Women in Aerospace and the International Academy of Astronautics.

EMILIE CLEMMENS was an NRC Christine Mirzayan Science & Technology Policy Graduate Fellow at the Space Studies Board during the fall of 2004. Emilie earned a Ph.D. in bioengineering in December 2003 from the University of Washington and a B.S. in chemical engineering from the University of Kentucky. Her dissertation research was aimed at understanding molecular-level differences between cardiac and skeletal muscles, and she engineered a system to measure in vitro muscle protein mechanics. Dr. Clemmens is also the co-founder of the Forum on Science Ethics & Policy, which is a new organization dedicated to promoting dialogue in the Seattle area between scientists, policy experts, legislators, and the general public on timely issues concerning the ethics and policy of scientific research.

AMANDA SHARP, summer undergraduate intern research assistant, is a rising senior at Harvard University in Cambridge, Massachusetts. She is currently pursuing a bachelor's degree in physics, but her courses have included significant work in astronomy and math. Her undergraduate research work has included modeling the atmospheric profiles of extrasolar giant planets and laser ablation inductively coupled plasma mass spectrometry.

CARMELA J. CHAMBERLAIN has worked for the National Academies since 1974. She started as a senior project assistant in the Institute for Laboratory Animals for Research (ILAR), which is now a board in the Division on Earth and Life Sciences (DELS), where she worked for 2 years before transferring to the Space Science Board, which is now the Space Studies Board.

CATHERINE A. GRUBER is an assistant editor with the Space Studies Board. She joined SSB as a senior program assistant in 1995. Ms. Gruber first came to the NRC in 1988 as a senior secretary for the Computer Science and Telecommunications Board and has also worked as an outreach assistant for the National Academy of Sciences-Smithsonian Institution's National Science Resources Center. She was a research assistant (chemist) in the National Institute of Mental Health's Laboratory of Cell Biology for 2 years. She has a B.A. in natural science from St. Mary's College of Maryland.

B

Recommendations from Two Previous NRC Reports on Forward Contamination

TABLE B.1 Recommendations from Two Previous National Research Council (NRC) Reports on Forward Contamination of Planetary Bodies

Category	Recommendation	Report
Pre-Launch and Pre-Cursor Studies	Techniques for assessing the existence of microorganisms have advanced dramatically since pre-Viking days. These advances will have a strong impact both on bioburden assessment procedures and on future life-detection experiments. New methods have been developed with increasingly greater sensitivity and specificity. **The task group strongly recommends that efforts be made to explore current analytical methods for use in bioburden assessment and inventory procedures before spacecraft assembly and launch.**	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 5.
	The task group recommends a number of studies that would improve knowledge of Europa and that would better define the issues related to minimization of forward contamination. These include studies on the following topics: • Ecology of clean room and spacecraft-assembly areas, with emphasis on extremophiles such as radiation-resistant microbes; • Detailed comparisons of bioload assay methods; • Desiccation- and radiation-resistant microbes that may contaminate spacecraft during assembly; • Autotroph detection techniques; and • Europa's surface environment and its hydrologic and tectonic cycles.	*Preventing the Forward Contamination of Europa*, NRC, SSB, 2000, p. 5.

continued

TABLE B.1 Continued

Category	Recommendation	Report
Sterilization and Pre-sterilization	Based on the MESUR [Mars Environmental Survey, later renamed Mars Pathfinder] group's consensus and the task group's agreement with it, the task group makes the following recommendations for control of forward contamination, each tied to specific mission objectives.	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 7.
	Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined. Viking technology may be adequate, but requirements will undoubtedly be driven by the nature and sensitivity of the particular experiments. The objective of this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter and/or microorganisms deposited at the landing site.	
	Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures—such as clean-room assembly and cleaning of all components—for bioload reduction, but such spacecraft need not be sterilized.	
	The task group's recommendation to reduce bioload on all spacecraft and to sterilize those spacecraft used in life-detection missions assumes the use of Viking procedures. However, **the task group recommends that the Viking protocols for assessment of spacecraft bioloads be upgraded to include state-of-the-art methods for the determination of bioload.** It is critical that methods for assessing bioload be compatible with methods used to detect life, with methods for both assessment and detection reflecting the same limits and sensitivity. Data on bioloads of Viking components and spacecraft are not relevant to current life-detection procedures. Modern methods of bioburden assessment should be developed for and applied to spacecraft destined for future Mars missions, especially those carrying in situ extant life-detection experiments.	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 9.
	… the bioload of each Europa-bound spacecraft must be reduced to a sufficiently low level at launch that delivery of a viable organism to a subsurface ocean is precluded at a high level of probability. This approach allows mission planners to take advantage of the bioload reduction likely to occur en route, particularly while in Jupiter's radiation environment. One consequence of this view is that Europa must be protected from contamination for an open-ended period, until it can be demonstrated that no ocean exists or that no organisms are present. Thus, we need to be concerned that over a time scale on the order of 10 million to 100 million years (an approximate age for the surface of Europa), any contaminating material is likely to be carried into the deep ice crust or into the underlying ocean.	*Preventing the Forward Contamination of Europa*, NRC, SSB, 2000, p. 2.
	The task group therefore recommends the following standard: for every mission to Europa, the probability of contaminating a europan ocean with a viable terrestrial organism at any time in the future should be less than 10^{-4} per mission. This standard calls for explicit calculation of the probability of contamination posed by each particular mission. It allows spacecraft designers to take advantage of the bioload reduction that occurs from radiation in the jovian environment. The value of 10^{-4} was chosen because of its historical precedents in the planetary protection resolutions issued by COSPAR.	*Preventing the Forward Contamination of Europa*, NRC, SSB, 2000, p. 22.
	NASA must devise a method for carrying out this calculation. An example of how such a calculation might be done is given in Appendix A. The task group's suggested methodology subdivides the bioload into common microorganisms, spores, radiation-resistant spores, and highly radiation-resistant nonspore microorganisms (e.g., *Deinococcus radiodurans;* see Chapter 3).	*Preventing the Forward Contamination of Europa*, NRC, SSB, 2000, p. 22.

TABLE B.1 Continued

Category	Recommendation	Report
Piloted Versus Unpiloted Missions	**The task group strongly recommends that a sequence of unpiloted missions to Mars be undertaken well in advance of a piloted mission.** Any future changes in recommendations to ensure planetary protection, especially for piloted or sample return missions, will depend on the acquisition of new data.	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 8.
	With regard to these missions, the task group recommends that a broad spectrum of martian sites be examined, with emphasis on measurements that provide data most likely to contribute to models that provide for a better understanding of the probability of life on Mars and where best to go to find it.	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 8.
Societal Issues	A substantial number of active national and international organizations are on the alert for environmental abuse. There is every reason to take seriously the concern (already expressed in some cases) about contamination of Mars and almost certainly about the issue of back contamination of Earth by martian samples. Although public concern over such issues is often sincere and productive, it at times becomes distorted and exaggerated in the media, leading to public misunderstanding and opposition. **The task group recommends that NASA inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return.**	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 9.
Legal Issues	There are also legal issues that must be addressed, involving international restrictions as well as federal, state, and local statutes that may come into play. There are currently no binding international agreements concerning forward or back contamination. **The task group recommends as essential that efforts be made (1) to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and (2) to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars. Furthermore, the task group recommends that NASA make a strong effort to obtain international agreement for a planetary protection policy.**	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, pp. 9-10.
NASA Planetary Protection Program	Although a planetary protection officer currently exists at NASA, there is no budgeted program (as there was during the Viking Program) to implement needed planetary protection research, a public education program, examination of legal and international issues, and the like. **The task group recommends that NASA redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the recommendations made in this report.**	*Biological Contamination of Mars: Issues and Recommendations,* NRC, SSB, 1992, p. 10.

C

Summary of Procedures Currently Used to Assess Bioburden in Spacecraft Assembly Clean Rooms and on Spacecraft

NASA NPR 5340.1C is a compilation of procedures for the microbiological examination and assessment of space hardware and associated environments that were developed to meet the requirements of NASA planetary protection and space and life science programs.[1] Although discussions are currently under way to develop new assay techniques based on molecular methods, the requirements for currently used procedures are still based on Viking-era culture techniques. Until newer molecular assays and updated procedures are adopted officially, NPR 5340.1C remains in force.

This appendix summarizes the current uniform microbiological assay procedures required for use in assessing (1) the degree of microbiological contamination of intramural environments where spacecraft hardware is assembled, tested, and launched; and (2) the level of microbial contamination on spacecraft hardware in relation to the known or anticipated environments of the target planets. Deviation from these procedures is not permitted unless written approval from NASA's planetary protection officer is granted.

All operations summarized below that involve the manipulation of sterile items and sample processing must be performed in laminar flow environments meeting Class-100 air cleanliness requirements of Federal Standard 209B. These procedures are designed primarily for the detection and enumeration of heterotrophic, mesophilic, anaerobic, and anaerobic microorganisms. Procedures for the detection of other microorganisms (e.g., psychrophiles and thermophiles) are also included to meet the needs of specific missions. Since the days of Viking, spore-forming bacteria that remain after heat shocking have been the standard measure for enumerating microbial contamination for planetary protection purposes. Although microbial assays described below are based on culture methods, only those with a heat-shocking regime are used specifically for detection and enumeration of spores on spacecraft hardware as stipulated by planetary protection requirements.

ASSAYS TO SAMPLE THE CLEAN-ROOM ENVIRONMENT

The clean-room environment at the assembly, test, and launch facilities is assessed in a number of ways in order to test for various categories of contaminants ranging from airborne contaminants accumulating on surfaces, to contaminants on environmental surfaces, and intramural air.

[1]The complete version of NASA NPR 5340.1C is available at <www.planetaryprotection.nasa.gov>.

• *Airborne microbial contamination* accumulating on surfaces is assessed by methods using fallout strips and ribbons placed throughout a site and collected at intervals. Sample strips are collected, immersed in test tubes, sonicated and heat shocked, and rapidly cooled before portions of the liquid from each tube are transferred onto petri plates for incubation at specified temperatures for specified periods under specified conditions. Plates are aerobically or anaerobically incubated and scanned at 24 and 48 hours to detect any growth. Final colony counts are made at 72 hours. In certain cases, plates are incubated for longer periods under varied conditions to encourage selection of psychrophilic or thermophilic organisms. If required, assay conditions may be modified and directed toward particular groups of microorganisms (e.g., aerobes, aerobic spores, or anaerobic spores). Upon completion of colony counts, results are expressed as number of microorganisms per strip or ribbon, and are reported by general categories of microorganisms (e.g., aerobes, aerobic spores, or anaerobic spores).

• *Microbiological sampling of environmental surfaces* is accomplished in several ways. For example, a plate method is used to sample flat, rigid surfaces (e.g., work tables and floors) and nonrigid surfaces (e.g., clothing or packaging materials). The plate method uses preprepared agar plates that are impressed onto the surfaces to be sampled and then incubated under appropriate conditions. Plates are scanned for visible growth at 24 and 48 hours, with final counts taken after 72 hours. If required, plates may be incubated for longer periods under specified conditions to encourage selection of particular groups of microbes. Various swab-rinse methods are also used to assay environmental surfaces, as are wipe-assay methods. As with the aforementioned methods, these involve the use of specific collection materials (cotton swabs or sterile cloths) to sample surfaces, followed by immersion of the sampling material in a solution and then sonication, subsampling, and incubation on petri plates under specified conditions and times. Plates are scanned for growth at appropriate intervals, and results are expressed as microorganisms per square centimeter of surface.

• *Microbiological sampling of intramural air* within a facility is conducted by using slit sample agar impaction devices or membrane filter field monitors. In both cases, specified volumes of air are collected on agar plates or membrane filters, and the plates and plated materials are then incubated for specified periods of time. Plates are scanned for growth at appropriate intervals, and results are reported as the number of airborne viable particles per liter of air.

SAMPLING OF SPACECRAFT HARDWARE TO DETERMINE CLEANLINESS AND BIOBURDEN

Sampling methods for spacecraft hardware use a variety of assays depending on the size, shape, and irregularity of the hardware being sampled. The same assay methods are used to assess bioburden during the assembly, test, and launch operations by the mission team and later by the designee of the Planetary Protection Office to certify and validate required bioburden and cleanliness levels.

• *Surfaces of piece-parts (subcomponent-sized hardware pieces)* are sampled by placing the entire part into a flask with sterile rinse solution, sonicating the flask to suspend contaminants in the solution, heat shocking, subsampling the liquid, and plating on petri dishes. Incubation under specified conditions is followed by making counts of microbial colonies at appropriate intervals of time. Results are expressed as the number of microorganisms per piece-part, based on the measured total surface area of the part. Suitable sterility control checks are also required on the entire assay procedure. In addition, as part of the process, an inhibitory test is performed on each type of piece-part after sterilization with dry heat. Spores of *Bacillus subtilis var. niger* are added to a flask with the part, followed by sonication, removal of the part, and incubation of the flask for 72 hours. A viability check on the spore inoculum is done after incubation.

• *Components, boards, modules, subsystems, systems, and landing capsules* are sampled by using swab-rinse or wipe-rinse methods, or both, depending on the area sampled. This is followed by sonication, heat shocking, plating, and culturing for 72 hours. In addition, hardware design provides for the inclusion of detachable strips on the surface of each flight item. A detachable strip assay method is also done as needed by using the method described earlier for assaying airborne contaminants accumulating on strips.

D

History of Recommended Values for Probability of Growth

Historically, the approach used to evaluate planetary protection requirements for spacecraft sent to Mars depended on efforts to estimate the probability P_g that Earth microorganisms would grow in the martian environment (see Chapter 2). As shown in Figure D.1, estimates of P_g varied by ten orders of magnitude during the period from 1964 to 1978. As the committee discusses in Chapter 5, the scientific basis for estimates of P_g—knowledge about the capability of Earth microorganisms to survive and propagate on Mars—remains strongly limited.

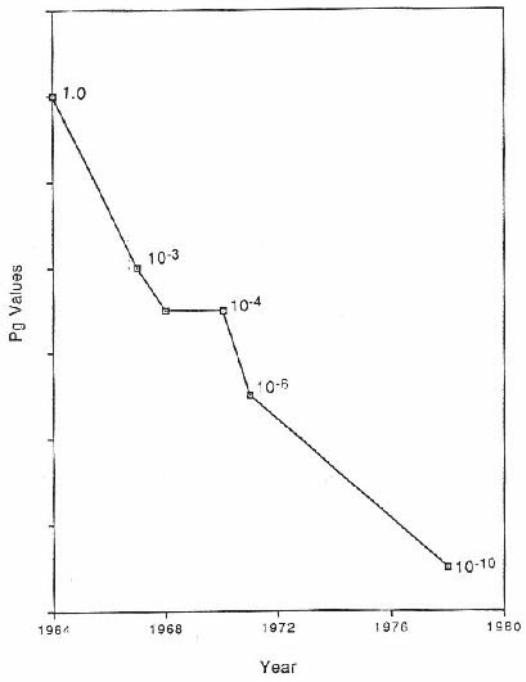


FIGURE D.1 Estimates of the probability of growth (P_g) of Earth microorganisms on the planet Mars. SOURCE: H.P. Klein. 1992. History of P_g. Pp. 41-52 in *Planetary Protection Issues for the MESUR Mission: Probability of Growth (P_g)*, H.P. Klein, ed. NASA CP 3167. NASA, Washington, D.C.

E

Approaches to Bioburden Reduction for Lander Missions to Mars

TABLE E.1 Approaches to Bioburden Reduction for Lander Missions to Mars

Mission	Type	Year	Viking Pre-Sterilization	Viking Post-Sterilization	Other Approaches
Viking Landers	Life-Detection Instruments COSPAR Category IVb	1975-1976	Assembly was done in class-100,000 clean rooms.[a] Thousands of microbial assays were conducted during assembly, which established an average spore burden per square meter of less than 300. The total burden on the lander surface (i.e., the exposed exterior and those parts of the interior communicating directly with the exterior) was less than 300,000. *Bacillus subtilis*, the spore-forming microbe, was used as an indicator organism in the microbiological assays because of its resistance to heat, desiccation, and radiation.	After assembly in clean rooms and application of microbial assays, the landers were sealed in bioshields. Bioburden was further reduced through dry heating at humidity of 1.3 mg/l. A minimum temperature of 111.7°C was maintained for 30 h and much of the lander was subjected to higher temperature over a longer time period. The efficacy of the sterilization procedure was estimated indirectly on the basis of the known heat-survival characteristics of *B. subtilis* and was credited with reducing the lander's bioburden by a factor of 10^4.[b]	

continued

TABLE E.1 Continued

Mission	Type	Year	Viking Pre-Sterilization	Viking Post-Sterilization	Other Approaches
Mars Pathfinder Lander/ Rover	No Life-Detection Instruments COSPAR Category IVa	1996	Assembly was done in class-100,000 clean rooms. All exposed surfaces were cleaned by alcohol wiping or dry heat, except that Sojourner rover was precision cleaned by Freon vapor degassing. Pre- and post-process microbial burden tests were performed for components that had not maintained humidity controls during heating. During early assembly, test, and launch operations at JPL, flight hardware was periodically cleaned by alcohol wiping. Final aerobic spore density and total burden were calculated at 14.9 spores m^{-2} and 2.9×10^4 spores, respectively.[c]	N/A	At Kennedy Space Center, SAEF-2 clean-room and strict personnel garment requirements were used; ground-support equipment and nonflight equipment were alcohol wiped.
Beagle 2 Lander/ Rover	Life-Detection Instruments COSPAR Category IVa+	2003	The probe was assembled aseptically in a class-100 clean room. Some components were sterilized with dry heat. Gamma irradiation, gas plasma, and alcohol wipes were used to sterilize technologies that were susceptible to heat. The assembly facilities were monitored at Viking levels for microbial presence, and assays were conducted based on NASA protocols.[d]	The completed probe was sealed in a front and back shield and bioseal and protected from recontamination by a HEPA filter. The probe was fitted with the Mars Express spacecraft in Toulouse, France, at a class-100,000 clean room and assembled and integrated with the launch vehicle in Baikonaur, also in a class-100,000 clean room. The external area of the Beagle was cleaned at each location.[d]	Established policies and procedures for planetary protection were followed in the assembly facility, including training, apparel, monitoring of people and material flow into assembly area, cleaning, cleaning of hardware, and sterilization.

TABLE E.1 Continued

Mission	Type	Year	Viking Pre-Sterilization	Viking Post-Sterilization	Other Approaches
MER Landers/ Rovers	No Life-Detection Instruments COSPAR Category IVa	2004	The hardware design was compatible with swab assays and alcohol wipe cleaning. Hardware that could not tolerate alcohol (honeycomb structures, airbags, parachutes, solar arrays) was sterilized using dry heat. Heating was done at 110°C for 50 h and 125°C for 5 h for some components. Tubing elements were precision cleaned with an acid wash, Freon degreasing, and other cleaning methods. Enclosed electronic components were isolated from the Mars environment by high-efficiency particulate air (HEPA) filters. The spacecraft was assembled in a class-100,000 clean room. Hardware that was dry heat sterilized was double bagged for protection. Elements that were not cleaned were covered.[e]	N/A	Early planning allowed for more extensive use of dry heat.

[a]Class-100,000 clean rooms: rooms with air quality control to a maximum of 100,000 particles of 0.5 μm in diameter per cubic foot of air.
[b]NRC, *Preventing the Forward Contamination of Europa*, National Academy Press, Washington, D.C., 2000, p. 5.
[c]Jack Barengoltz. Microbial cleanliness of the Mars Pathfinder spacecraft. Pp. 242-248 in *Proceedings of 43rd Annual Technical Meeting Contamination Control.* Institute of Environmental Sciences, 1997.
[d]Andrew Spry, Open University, presentation to the NRC Committee on Preventing the Forward Contamination of Mars, February 27, 2004.
[e]Laura Newlin, Planetary Protection Lead for the MER Missions, Jet Propulsion Laboratory, discussion with the NRC Committee on Preventing the Forward Contamination of Mars, February 26, 2004; "Mars Exploration Rover Spacecraft Undergo Biological Testing and Cleaning Prior to June Launches," KSC Release No. 37-03, May 23, 2003, NASA John F. Kennedy Space Center.

F

Ambiguities in Geomorphic Interpretation: Martian Gullies

Geomorphic analysis has an important shortcoming as a potential indicator of the present three-dimensional distribution and state of subsurface water on Mars, in that its interpretations are often not unique and are therefore controversial. This problem is illustrated here by considering recent geomorphic analyses of martian gullies.

The fluvial appearance of martian gullies (Figure F.1) has been interpreted as evidence of recent and possibly contemporary discharges of liquid water from near-surface aquifers (Malin and Edgett, 2000). This discovery generated considerable interest in the potential use of such features to identify places where liquid water may be present at shallow depth. However, subsequent analyses of the available observational data, and consideration of the environmental challenges posed by the presence of near-surface liquid water, have raised significant doubts about the uniqueness and plausibility of the shallow aquifer hypothesis.

The key characteristics of the gullies are a fluvial-like morphology that incises the local terrain; a shallow depth of origin (~100 to 500 m) on topographic exposures (e.g., simple scarps, mesas, knobs, crater walls, and central peaks); an apparent youthful age ($\leq 10^7$ years) supported by their fresh appearance, superpositional relationship to other assumed transient landforms (e.g., sand dunes), and crater counts; a geographic distribution in both hemispheres that is restricted to latitudes between ~30° and 70°; and a preferential occurrence on poleward-facing slopes (Malin and Edgett, 2000). In addition, the gullies lack any obvious association with areas of past geothermal activity, such as Tharsis and Elysium (Gulick, 2001).

If the gullies were truly formed by the discharge of liquid water from shallow aquifers, this would imply a combination of geothermal heat flow, crustal thermal conductivity, and groundwater freezing temperature sufficient to reduce the local thickness of frozen ground by a factor of ~10 to 100 over the values generally expected to characterize the cryosphere at these latitudes (see Figure 4.1). Of these three variables, the one most likely to exhibit the greatest variability (when considered on a spatial scale of kilometers) is the planet's geothermal heat flow, which, in localized areas, might easily exceed the estimated global mean by as much as several orders of magnitude (assuming the presence of a local igneous intrusion). Although an enhanced local heat flow might explain the origin of some gullies (Malin and Edgett, 2000; Gulick, 2001), it fails to explain three of their most notable characteristics: their observed latitudinal distribution, their preferential occurrence on poleward-facing slopes, and—most important—their lack of any obvious association with recognized regions of past geothermal activity (like Tharsis and Elysium).

Another explanation for the possible occurrence of near-surface liquid water is the potential presence of potent freezing-point depressing salts, such as $CaCl_2$ and $MgCl_2$, which could lower the freezing point by as much

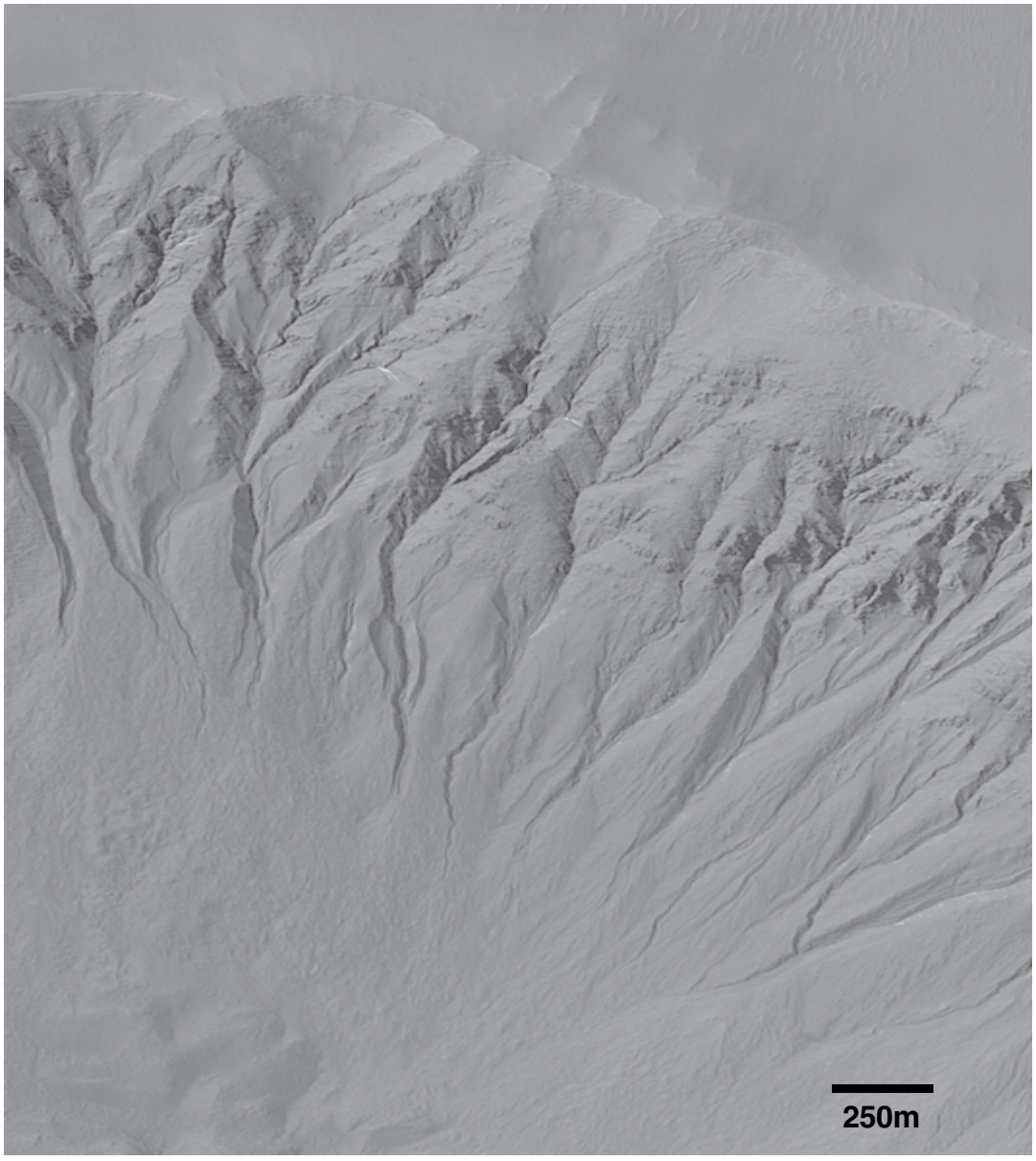


FIGURE F.1 Example of gullies present on the interior wall of a crater at 37.3°, 168°W. SOURCE: Malin and Edgett (2000).

as 60 K (to ~210 K) (Brass, 1980; Clark and Van Hart, 1981; Knauth and Burt, 2002). But the involvement of brines in the origin of the gullies is difficult to reconcile with the lack of any visible evidence of evaporite deposits associated with these features and with the inability of brines to explain either the latitudinal distribution or the poleward-facing orientation of the gullies.

Mellon and Phillips (2001) have advanced another hypothesis. They argue that the occurrence of shallow aquifers can be explained by the presence of thick mantles of extremely low thermal conductivity regolith, creating a sufficiently large geothermal gradient that the temperature of the local crust is raised above the melting point

within just a few hundred meters of the surface. However, for the mantle to retain its low conductivity, it must remain ice free, a condition that requires that the aquifer be diffusively isolated from the overlying mantle by an essentially impervious barrier. This requirement is imposed by the fact that, given the diffusive characteristics of most reasonable geologic materials, sufficient water vapor will diffuse from the aquifer and condense in the mantle to saturate its pore volume with ice in a geologically short period of time (~10^3 to 10^7 years; Clifford, 1995). The resulting increase in the thermal conductivity will be sufficient to cause any near-surface aquifer to freeze. The requirement for diffusive isolation appears inconsistent with the widespread occurrence of gullies in such highly disrupted terrains as the interior walls and central peaks of large craters.

Given the difficulty of reconciling the shallow aquifer hypothesis with both plausible environmental conditions and the need to explain the various enigmatic characteristics of the gullies (e.g., their restriction to mid latitudes to high latitudes and preferential occurrence on poleward-facing slopes), a variety of alternative explanations have been proposed, including erosion by liquid CO_2 (Musslewhite et al., 2001) and dry mass wasting (Treiman, 2003). But these alternatives face many of the same environmental challenges confronting the shallow aquifer hypothesis (e.g., Stewart and Nimmo, 2002; Heldmann and Mellon, 2004).

Advocates suggest that there is one explanation for the origin of the gullies that satisfies the most serious environmental and observational constraints. The martian obliquity is known to be chaotic on a timescale of ~10^7 years, varying from ~0° to 60° (Touma and Wisdom, 1993; Laskar and Robutel, 1993; Laskar et al., 2004). For obliquities ≥45°, the peak insolation on poleward-facing slopes at mid latitudes to high latitudes can yield summertime surface temperatures that easily exceed the melting point for continuous periods that range from hours to many months (Toon et al., 1980; Pathare and Paige, 1998; Costard et al., 2002). Under these conditions, large amounts of water ice are expected to sublime and melt from the summer polar ice cap, increasing the atmospheric vapor pressure of H_2O sufficiently to allow liquid water to flow readily across the surface. This input of vapor could also amplify the extent of polar warming by creating a transient and localized water vapor greenhouse (Pathare and Paige, 1998). Under such conditions, formerly stable near-surface ice deposits (whether in the form of snow or shallow subsurface ice) could conceivably melt and produce sufficient runoff to form the gullies (Paige, 2002; Costard et al., 2002; Stewart and Nimmo, 2002; Christensen, 2003). Indeed, it has been argued that the local condensation and melting of atmospheric H_2O may be contributing to the development of the gullies even today (Hecht, 2002), a mechanism perhaps greatly enhanced at times of high obliquity. However, the high obliquity model is not without its critics. For example, Mellon and Phillips (2001) have argued that at high obliquity the atmospheric vapor pressure of H_2O is so low that ground ice will sublime from the regolith before any liquid melt is produced. In addition, Treiman (2003) and Heldmann and Mellon (2004) have noted that while most gullies do show a general poleward orientation, there are many exceptions that appear inconsistent with a high-obliquity origin.

The preceding analysis is by no means exhaustive, nor does it conclusively refute the viability of any suggested mechanism for the origin of the gullies. But it does demonstrate the enormous technical difficulties and interpretive uncertainties associated with understanding these landforms—uncertainties that for now preclude the unambiguous interpretation of not only the gullies, but also most other potential geomorphic indicators of the current distribution of subsurface H_2O as well (e.g., rampart craters, softened terrain, and patterned ground).

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G

Spacecraft Propellant and By-Products as Potential Contaminants

An important concern with respect to potential contamination is the release of propulsion exhaust products into the atmosphere and onto the surface of Mars, a process that necessarily occurs during any Mars entry, atmospheric flight/descent, and landing. The amount of exhaust products emitted can range from as little as a few kilograms (Pathfinder) to more than 30 metric tons (estimated for a human ascent stage). Two concerns arise: (1) Would contamination caused by the exhaust products of propulsion confound the measurement capabilities of a mission's science payload? (2) Could this contamination sufficiently affect the global environment to irreversibly alter or mask important atmospheric markers of natural conditions?

PROPULSION-RELATED CONTAMINATION—BACKGROUND

Over the 32-year period from 1971 through 2003, 16 landers were launched to Mars, 8 by NASA, 7 by the Soviet Union, and 1 by ESA. Of these landers, 12 entered the Mars atmosphere, and 6 landed successfully and sent back data (see Table 1.1). The most successful landers include Viking 1 and 2 (1975 launch), Pathfinder (1996), and the Mars Exploration Rovers (MERs A/B) Spirit and Opportunity (2003). All of these landers used some form of retrorocket propulsion to reduce or cancel their surface impact velocity. Viking used a liquid monopropellant to achieve a soft landing, and Pathfinder and the MERs used solid rocket motors to reduce their impact velocity to levels that could tolerate the use of airbags.

The first and only known assessment of landing site contamination from rocket exhaust gases was performed experimentally, during the development of the Viking lander, in a specially designed test chamber using a full-scale, one-third model of the Viking lander, including a lander segment of one descent engine and one landing leg (Husted et al., 1977). The rocket exhaust gas testing was conducted in a Mars environment test chamber and simulated the actual descent motion of the vehicle and its landing on a simulated soil surface. Sample test cups holding soil samples were placed in strategic locations in the test chamber soil to collect any contamination from the rocket exhaust gas. Both of the test samples and the chamber gas were analyzed for contamination that may have resulted from the firing of the descent engine during multiple test runs. During the assessment, engineers changed the descent engine nozzle design (which was subsequently used on the Viking landers) to diffuse the exhaust and reduce soil erosion as the lander approached the surface. The results of chemical analysis of the soil samples showed measurable amounts of hydrogen cyanide (HCN), which were traced to aniline impurities in the Mil-Spec fuel. Switching to a purified hydrazine fuel eliminated the unwanted HCN, and that fuel has since been

the standard for all subsequent Mars soft landers to date, although tests also showed that the purified hydrazine would contaminate Mars landing sites with ammonia (50 to 500 ppm), N_2 (5 to 50 ppm), and possibly a small amount of water (quantity not measured). The large amounts of ammonia trapped in the soil would make interpretation of organic analyses more difficult.[1] The results of the rocket exhaust assessment also led to the conclusion that, for the Viking mission, the combined effects of plume gases, surface heating, surface erosion, and gas composition resulting from the use of retrorockets would not interfere with the planned biology investigation (Husted et al., 1977).

The rocket exhaust study conducted for the Viking mission also addressed gas dissipation from the soil after landing (actually release and diffusion), because measuring the chemical composition of the atmosphere (especially the presence of less abundant species to accuracies of 100 ppm) was another mission objective. The Viking Molecular Team set as criterion that exhaust gas contamination at a concentration above 10 ppm be permitted to exist for no more than 2 days after landing. That period was considered to be sufficient for diffusion of the plume gases (actually calculated to be 2.7 days, but any minor wind or air movement will decrease the amount of time) (Husted et al., 1977).

No subsequent comprehensive analyses for retrorocket contamination have been performed. The Viking assessment results have served as the ad hoc basis for all subsequent NASA Mars soft-lander missions, that is, Mars Polar Lander, Mars '01 (not flown), and Phoenix (in development). Furthermore, the use of solid retrorockets on Pathfinder and MER A/B, which emit very different exhaust products (including aluminum oxide and carbonaceous compounds from pyrolysis of the ethylene propolyne diene monomer rubber case insulation), has not been assessed as a potential source of contamination because those landers came to rest quite a distance from the location of the terminal retrorocket burn.

POTENTIAL CONTAMINANTS FROM FUTURE MISSIONS

NASA's Mars Exploration Program has planned a series of landing missions that will follow the Phoenix Lander mission in 2007 (see Chapter 3). The Mars Science Laboratory (MSL) mission in 2009 will be a nuclear-powered rover with an order-of-magnitude more payload (50 kg, 10 instruments) and enhanced capability as compared with the MERs. MSL will employ a unique "sky-crane" landing system that will allow it to lower the rover on a tether to the surface while hovering on retrorockets and then fly away from the landing site (see Figure G.1). Follow-on landers such as the Astrobiology Field Laboratory and Mars Sample Return (MSR) missions will most likely use this sky-crane landing system. The sky crane is expected to use hydrazine retrorockets as did Viking, but it may have throttled engines rather than pulsed engines, which would reduce potential disturbances of soil.[2] Nonetheless, the sky crane will require larger loads of hydrazine propellant for their payloads (200 kg compared with 85 kg for Viking) and will at least temporarily contaminate the atmosphere surrounding the landing site.

The first stage of the planned Mars Ascent Vehicle (MAV) of MSR missions poses another possibility for contamination of the rocket site and atmosphere. Current designs use a solid motor first stage that ignites at the surface and burns to an altitude of approximately 15 km. Whether or not the resultant contamination would be tolerable, given that the sample would already have been collected, would likely depend on whether any subsequent surface investigations were planned to be conducted after the ascent stage launch and on the nature of the atmospheric contamination by the rocket during launch.

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[1]According to Husted et al., "The presence of ammonia complicates both the experiment preparation for Martian chemical analysis and the interpretation of the returned data. The primary concern is the potential reaction of ammonia with simple organics to form nitrogen-containing organics which would add to the difficulty in interpretation. Further, these new compounds could be of a biological nature and of the type considered primary for a life searching mission" (Husted et al., 1977, p. 22).

[2]Soil disturbances are what one would expect from the blast of the descent engine exhausts, that is, dust and small particles becoming airborne and surface excavation, among other factors. The exhaust from pulsed engines can be much stronger than that from continuously burning engines, which can be throttled to lower thrust levels.

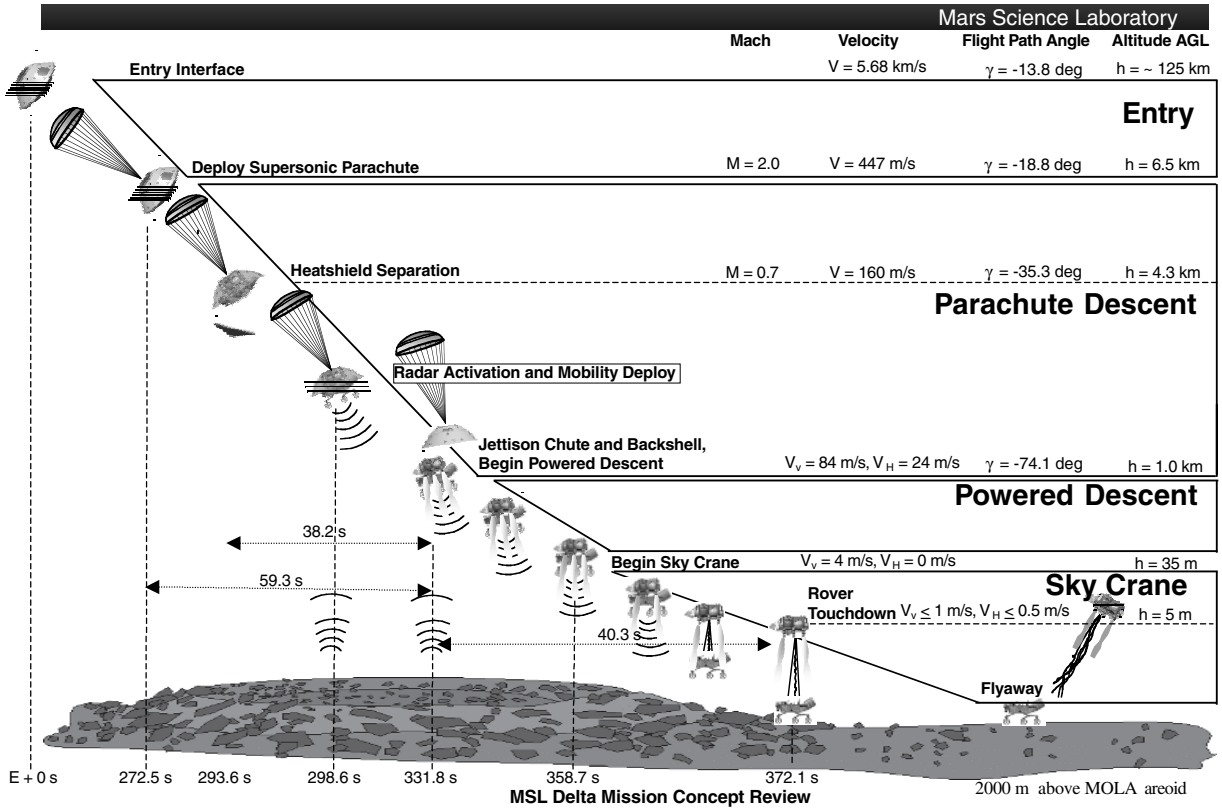

FIGURE G.1 Mars Science Laboratory nominal entry, descent, and landing using a sky crane for terminal descent. SOURCE: Mars Science Laboratory Project Office, Jet Propulsion Laboratory, July 2004.

Finally, various concepts for future missions within the Mars Exploration Program include significantly more capable long-range exploration missions involving, for example, robotic outposts with reusable regional transport systems (e.g., Mars airplanes) and human missions to Mars (see Chapter 2). For such missions, a much more capable propulsion system is desired in order to have manageable propellant loads. One system that appears favorable and within technology development capabilities would use liquid oxygen (LOX)-methane (CH_4) propellants in a cryogenic system that could double the payload launched from Mars (using the same propellant mass) as compared with the payload mass that can be launched by using hydrazine propellant. The LOX/CH_4 system is also of interest because the propellant can be manufactured in situ in Mars's atmosphere (by electrolysis of constituents there with hydrogen feedstock). Exhaust products from this system are primarily CO, CO_2, and H_2O, gases that in reasonable quantities will not be considered contaminants of the Mars atmosphere. However, accidental release of the produced methane fuel might be another matter (e.g., confounding local atmospheric trace gas measurements), especially given the quantities of propellants required for human missions (e.g., >6 metric tons of methane for a Mars ascent stage for humans; MSFC, 1991).

Table G.1 presents a brief summary of the amount and types of exhaust products that could result from the use of particular propellants for future lander missions to Mars. As is apparent from these data, the combination propellants (involving use of more than one kind of propellant) all emit water and various other compounds, usually including carbon monoxide and carbon dioxide. The committee is not aware of any analyses or planned studies of the potential for contamination by exhaust products that might be associated with the types of future missions discussed for NASA's Mars Exploration Program.

TABLE G.1 Examples of Possible Amounts and Composition of Mars Surface Mission Propellant Exhaust Products

Missions >	Phoenix	MSL	MSR Lander	MSR MAV	Mars Airplane	Ascent Stage for Humans
Propellants >	N_2H_4	N_2H_4	N_2H_4/N_2O_4	$NH_4ClO/Al/HTPB$	MMH/N_2O_4	CH_4/O_2
Typical > Propellant > Masses (kg) >	35	250	250	160	40	30000
Exhaust Products (kg)						
H				0		2
H_2	2	17	7	3	1	122
HO				0		26
H_2O			90	15	9	13533
O						0
O_2						5
N_2	21	152	153	14	17	
NH_3	11	81	0			
CO				31	3	2762
CO_2				7	10	13551
Cl				0		
ClH				35		
Al_2O_3				54		

SOURCE: Data provided by J. Niehoff and G. Chew of SAIC using the Air Force Chemical Equilibrium Specific Impulse Code, a rocket exhaust products chemical equilibrium code, August 2004.

REFERENCES

Husted, R.R., I.D. Smith, and P.V. Fennessey. 1977. *Site Alteration Effects from Rocket Exhaust Impingement During a Simulated Viking Mars Landing*. NASA CR-2814. NASA, Washington, D.C., March.

MSFC (Marshall Space Flight Center) Technical Study Team. 1991. *Mars Transportation System*. Doc. No. 5-130-0-5. MSFC, Huntsville, Ala., March.

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Acronyms and Abbreviations

ATLO	assembly, test, and launch operations
ATP	adenosine triphosphate
CETEX	Committee on Contamination by Extraterrestrial Exploration
COSPAR	Committee on Space Research
DNA	deoxyribonucleic acid
EDL	entry, descent, landing
ESA	European Space Agency
EURECA	European Retrievable Carrier
GCM	general circulation model
GPR	ground-penetrating radar
GRS	gamma ray spectrometer
Gya	billion years ago
ICSU	International Council for Science
JPL	Jet Propulsion Laboratory
LAL	limulus amebocyte lysate
LDEF	Long Duration Exposure Facility
MARSIS	Mars Advanced Radar for Subsurface and Ionospheric Sounding
MAV	Mars Ascent Vehicle
MEPAG	Mars Exploration Program Analysis Group
MER	Mars Exploration Rover
MOLA	Mars Observer Laser Altimeter

MRO	Mars Reconnaissance Orbiter
MSL	Mars Science Laboratory
MSPSG	Mars Science Program Synthesis Group
MSR	Mars Sample Return
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NHB	NASA Handbook
NMI	NASA Management Instruction
NPR	NASA Procedural Requirements
NRC	National Research Council
NSF	National Science Foundation
OCSSG	Organic Contamination Science Steering Group
PAH	polycyclic aromatic hydrocarbon
P_c	probability of contamination
PCR	polymerase chain reaction
P_g	probability of growth
PP	planetary protection
R&D	research and development
RNA	ribonucleic acid
rRNA	ribosomal RNA
SHARAD	shallow radar
SSB	Space Studies Board
TBD	to be determined
TES	thermal emission spectrometer
T-RFLP	terminal restriction fragment length polymorphism
UV	ultraviolet