Assessment of Planetary Protection Requirements for Mars Sample Return Missions

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Assessment of Planetary Protection Requirements for MARS SAMPLE RETURN MISSIONS

Committee on the Review of Planetary Protection Requirements for Mars Sample Return Missions

Space Studies Board

Division on Engineering and Physical Sciences

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Cover: Landforms in the Nilokeras Region of Mars and the crescent Earth (inset, *upper right*), as recorded by the High Resolution Imaging Science Experiment on the Mars Reconnaissance Orbiter. The main image, made by combining data recorded at near-infrared, red, and blue-green wavelengths, shows a swath of terrain approximately 1.2 kilometers in vertical extent. Image courtesy of NASA/JPL and the University of Arizona. The three inset images depict key phases in a sample return mission, including entry, descent, and landing (*left*); launch of the samples from the surface of Mars into orbit about the planet (*center*); and retrieval of the sample canister in orbit about Mars for return to Earth (*right*). Images courtesy of NASA/JPL. Cover design by Penny E. Margolskee.

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Preface

In a letter sent to Space Studies Board (SSB) Chair Lennard Fisk on February 6, 2008, S. Alan Stern, then NASA's associate administrator for the Science Mission Directorate (SMD), explained that substantial increases in knowledge of Mars had rekindled interest in a Mars sample return mission both by NASA and within the international space exploration community. In accordance with international treaty obligations, NASA maintains a planetary protection policy to avoid the biological contamination of other worlds as well as the potential for harmful effects on Earth from the return of extraterrestrial materials by spaceflight missions. Specific advice regarding the handling of samples returned to Earth from Mars is contained in the 1997 National Research Council (NRC) report *Mars Sample Return: Issues and Recommendations*.[1] As NASA and other space agencies prepare for a future Mars sample return mission, it is appropriate to review the findings of the 1997 report and to update its recommendations, taking into account current understanding of Mars's biological potential as well as ongoing improvements in biological, chemical, and physical sample analysis capabilities and technologies. These considerations led Dr. Stern to request an examination of the 1997 Mars report, with particular reference to the following topics:

- The potential for living entities to be included in samples returned from Mars;
- Scientific investigations that should be conducted to reduce uncertainty in the above assessment;
- The potential for large-scale effects on Earth's environment by any returned entity released to the environment;
- The status of technological measures that could be taken on a mission to prevent the inadvertent release of a returned sample into Earth's biosphere; and
- Criteria for intentional sample release, taking note of current and anticipated regulatory frameworks.

In response to this request, the ad hoc Committee on the Review of Planetary Protection Requirements for Mars Sample Return Missions was established in July 2008. The committee held its first meeting at Arizona State University in Tempe, Arizona, on August 12-14, 2008, to discuss the task it had been given and to hear relevant presentations. Additional discussions and presentations were heard at a second and final meeting held at the National Academy of Sciences in Washington, D.C., on September 8-10, 2008. A draft report was completed in early December and sent to external reviewers for commentary in mid-December. A new draft responding to the

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[1] National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

reviewers' comments was completed in late February 2009, and the report was approved in March 2009. The time between approval and release was spent in editing and production of the report.

The work of the committee was made easier thanks to the important help, advice, and comments provided by numerous individuals from a variety of public and private organizations. These include the following: Karen Buxbaum (NASA, Jet Propulsion Laboratory), Philip Christensen (Arizona State University), Catharine Conley (NASA, Science Mission Directorate), Michael J. Daly (Uniformed Services University of the Health Sciences), Paul Davies (Arizona State University), Noel Hinners (Lockheed Martin Astronautics, retired), Bruce M. Jakosky (University of Colorado), Gigi Kwik-Gronvall (University of Pittsburgh Medical Center), James Nienow (Valdosta State University), John Priscu (Montana State University), John D. Rummel (East Carolina University), and Steven Squyres (Cornell University).

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the 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 its 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 review comments and draft manuscript remain confidential to protect the integrity of the deliberative process.

The committee wishes to thank the following individuals for their participation in the review of this report: Abigail Allwood, Jet Propulsion Laboratory; Michael H. Carr, U.S. Geological Survey (retired); Aaron Cohen, Texas A&M University; Kenneth H. Nealson, University of Southern California; Norman R. Pace, University of Colorado; Stefan Wagener, Canadian Science Centre for Human and Animal Health; and G.J. Wasserburg, California Institute of Technology.

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 Peter M. Banks, Astrolabe Venture Partners. Appointed by the NRC, he 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.

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Summary

NASA maintains a planetary protection policy to avoid the forward biological contamination of other worlds by terrestrial organisms, and back biological contamination of Earth from the return of extraterrestrial materials by spaceflight missions. Forward-contamination issues related to Mars missions were addressed in a 2006 report of the National Research Council's (NRC's) Space Studies Board (SSB), *Preventing the Forward Contamination of Mars*.[1] However, it has been more than 10 years since back-contamination issues were last examined.

Driven by a renewed interest in Mars sample return missions, this report reviews, updates, and replaces the planetary protection conclusions and recommendations contained in the NRC's 1997 report *Mars Sample Return: Issues and Recommendations*.[2] It is the understanding of the Committee on the Review of Planetary Protection Requirements for Mars Sample Return Missions that its conclusions and recommendations will be developed at the tactical level by subsequent groups specifically charged with the development of implementable protocols for the collection, handling, transfer, quarantine, and release of martian samples. This is the approach that was taken by NASA after its receipt of the 1997 Mars report. Indeed, the development of broad strategic guidelines by SSB committees and the subsequent development of tactical plans for their implementation by NASA committees is a general approach that has served the space-science community well for most of the past 50 years.

The specific issues addressed in this report include the following:

- The potential for living entities to be included in samples returned from Mars;
- Scientific investigations that should be conducted to reduce uncertainty in the above assessment;
- The potential for large-scale effects on Earth's environment by any returned entity released to the environment;
- Criteria for intentional sample release, taking note of current and anticipated regulatory frameworks; and
- The status of technological measures that could be taken on a mission to prevent the inadvertent release of a returned sample into Earth's biosphere.

IMPORTANCE OF MARS SAMPLE RETURN

A sample-return mission is acknowledged to be a major next step in the exploration of Mars because it can address so many high-priority science goals. The NRC's 2003 solar system exploration decadal survey, for example, highlighted three areas where unambiguous answers to key science issues are unlikely without a sample return mission:[3]

- The search for life;
- Geochemical studies and age dating; and
- Understanding of climate and coupled atmosphere-surface-interior processes.

Returning samples to Earth is desirable for a number of reasons, including the following:

- Complex sample-preparation issues relating to some high-priority activities are more readily tackled in terrestrial laboratories than they are by robotic means on Mars;
- Instrumentation that is not amenable to spacecraft application because of its bulk, mass, or power requirements can be used on Earth to analyze samples; and
- A greater diversity of instruments can be used on Earth to study samples than can be packaged to fit within the confines of any one robotic spacecraft or series of spacecraft, including instruments that were not available when the sample-return mission was launched.

REPORT ORGANIZATION

Since the purpose of this document is to revise, update, and replace the NRC's 1997 report *Mars Sample Return: Issues and Recommendations*, it is most logical to organize it around the basic question, What has changed since the release of the 1997 report?

Changes is scientific understanding can be summarized in the following manner:

- New insights on the roles played by surface and subsurface water throughout martian history and the potential for habitable environments on Mars—Chapter 2;
- Advances in microbial ecology that illuminate the limits of adaptability of life on Earth—Chapter 3;
- New understanding of the physical and chemical mechanisms by which evidence of life might be preserved on Mars and how that life might be detected in martian samples—Chapter 4; and
- New understanding of pathogenesis and the nature of biological epidemics, as well as additional insights as to the possibility that viable martian organisms might be transported to Earth by meteorites—Chapter 5.

The changes in the technical and/or policy environment can be organized as follows:

- A significant expansion of the size of the Mars exploration community and broadening of the scope of mission activities by both traditional and new space powers—Chapter 2;
- Greater societal awareness of the potential for technical activities to cause harmful changes in the global environment—Chapter 5;
- The de facto internationalization of a Mars sample return mission and subsequent sample-handling, sample-processing, sample-analysis, and sample-archiving policies—Chapter 6;
- The drafting and publication by NASA, with the assistance of international partners, of initial Mars sample-handling and biohazard-testing protocols based on the recommendations in the NRC's 1997 Mars report—Chapter 6;
- The development of nondestructive methods of analysis that can be used to map the microscale spatial distribution of minerals and biological elements in samples—Chapter 6; and
- The proliferation of biocontainment facilities driven by biosecurity concerns and associated changes in public policy and with public acceptance of such facilities—Chapter 7; and
- Lessons learned about the practical and logistical aspects of Mars sample return from experience with the Genesis and Stardust missions as well as experience gained from the planning for and commissioning of new biocontainment facilities—Chapter 7.

CONCLUSIONS AND RECOMMENDATIONS

The committee's conclusions and recommendations are organized according to the task outlined in the charge it was given by NASA.

The Potential for Living Entities in Samples Returned from Mars

The assessment of martian habitability made by the authors of the NRC's 1997 Mars report led them to recommend that: "Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized" (p. 3).

The present committee finds that the knowledge gained from both orbital and landed missions conducted over the last decade, combined with findings from studies of martian meteorites, has enhanced the possibility that habitable environments were once widespread over the surface of Mars. In addition, the potential for modern habitable environments, both as transient surface environments and as stable habitats in the deep subsurface, is much better understood.

Understanding the range of environmental conditions to which terrestrial life has adapted has directly shaped current views of martian habitability and the potential for samples returned from Mars to contain evidence of life. A substantial and growing body of evidence shows that life not only is present but also frequently thrives under extreme environmental conditions. Consideration of advances in microbial ecology over the past decade led the committee to reach the following conclusions:

- Biological studies have continued to expand the known environmental limits for life and have led to the discovery of novel organisms and ecosystems on Earth;
- Some living species on Earth have been shown to survive under conditions of extreme radiation, subfreezing temperatures, high salinity, extremely high and low pH, and cycles of hydration to dehydration present on Mars today;
- The discovery, in deep subsurface environments on Earth, of microbial ecosystems that are able to survive on inorganic sources of energy has greatly enhanced the potential for chemoautotrophic life in subsurface environments on Mars; and
- Studies have confirmed the potential for the long-term viability of terrestrial microorganisms sequestered in deposits of some extreme terrestrial environments (e.g., ices and evaporates) that have high relevance for Mars exploration.

Advances in the knowledge of environmental conditions on Mars today and in the past, combined with advances in understanding of the environmental limits of life, reinforce the possibility that living entities could be present in samples returned from Mars. Therefore, the committee concurs with and expands on the 1997 recommendation that no uncontained martian materials should be returned to Earth unless sterilized.

Recommendation: *Based on current knowledge of past and present habitability of Mars, NASA should continue to maintain a strong and conservative program of planetary protection for Mars sample return. That is, samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.*

The Potential for Large-Scale Effects on Earth's Environment

A key issue of concern is the possibility that a putative martian organism inadvertently released from containment could produce large-scale negative pathogenic effects in humans, or could have a destructive impact on Earth's ecological systems or environments.

The committee concurs with the basic conclusion of the NRC's 1997 Mars study that the potential risks of large-scale effects arising from the intentional return of martian materials to Earth are primarily those associated with replicating biological entities, rather than toxic effects attributed to microbes, their cellular structures, or extracellular products. Therefore, the focus of attention should be placed on the potential for pathogenic-infectious diseases, or negative ecological effects on Earth's environments. Like the 1997 committee, the present committee finds that the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero.

A related issue concerns the natural introduction of martian materials to Earth's environment in the form of martian meteorites. Although exchanges of essentially unaltered crustal materials have occurred routinely throughout the history of Earth and Mars, it is not known whether a putative martian microorganism could survive ejection, transit, and impact delivery to Earth or would be sterilized by shock pressure heating during ejection or by radiation damage accumulated during transit. Likewise, it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life, based on present evidence.

Thus, the conclusion reached from assessment of large-scale effects resulting from intentional and natural sample return is that a conservative approach to both containment and test protocols remains the most appropriate response.

Scientific Investigations to Reduce Uncertainties

Uncertainties in the current assessment of martian habitability and the potential for the inclusion of living entities in samples returned from Mars might be reduced by continuing activities in the following general areas: spacecraft missions to Mars, combined with related laboratory, theoretical, and modeling activities; investigations of the ecological diversity and environmental extremes of terrestrial life; geobiological studies of both modern and ancient Mars-relevant environments on Earth, with particular emphasis on biosignature preservation; and studies relating to the interplanetary transport of viable organisms.

The committee finds that the following activities are particularly relevant to reducing uncertainties:

- Remote-sensing and in situ exploration of Mars with the goal of answering questions relating to martian habitability, including those concerned with the presence of water in surface and subsurface environments through time, the distribution of biogenic elements, and the availability of redox-based energy sources (e.g., those based on the oxidation of ferrous iron and reduced sulfur compounds);
- Studies of martian meteorites to help refine understanding of the history of interactions of Mars's rock-water-atmosphere system throughout the planet's history;
- Studies of the metabolic diversity and environmental limits of microbial life on Earth;
- Studies of the nature and potential for biosignature preservation in a wide range of Mars-analog materials on Earth;
- Investigations of the prolonged viability of microorganisms in geological materials;
- Evaluation of the impacts of post-depositional (diagenetic) processes (deep burial, impact shock, subfreezing temperatures) on the long-term retention of biosignatures in ancient geological materials;
- Determination of reliable criteria for the definitive identification of biosignatures in ancient materials;
- Assessment of the potential for impact-mediated interchanges of viable organisms between Earth and Mars;
- Development of laboratory-based and in situ analytical approaches for biosignature analysis.

Criteria for Intentional Sample Release

There is a broad consensus in the scientific community that samples collected on Mars and returned to Earth must be contained and treated as potentially biologically hazardous until they are declared safe for release from containment by applying recommended protocols, including rigorous physical and chemical characterization, life detection analyses, and biohazard testing. It is important to emphasize that the high level of containment recommended for the handling and testing of martian samples is based on a deliberate decision to adopt a conservative approach to planetary protection and is not because of the anticipated nature of pristine martian materials or organisms. If anything, however, the discoveries over the past decade about environmental conditions on Mars today and in the past and about terrestrial extremophiles have supported an enhanced potential for the presence of liquid water habitats and, perhaps, microbial life on Mars. Thus it is appropriate to continue this conservative approach.

A factor that could potentially complicate the policies and protocols relating to sample containment and biohazard evaluation is the de facto internationalization of a Mars sample return mission. All serious planning for Mars sample return is founded on the premise that the scope, complexity, and cost of such a mission are beyond the likely resources of any one space agency. Although no major issues have arisen to date, the international interest in of Mars sample return raises the possibility that differences in national policies and legal frameworks of concerned parties might complicate issues relating to sample quarantine and biohazard certification.

Changes to the requirements for sample containment or criteria for sample release were issues of concern in the NRC's 1997 report *Mars Sample Return,* which recommended that: "The planetary protection measures adopted for the first Mars sample-return mission should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent oversight body" (p. 4). The present committee concurs with the spirit of that recommendation, with three provisos: first, that the protocols for sample containment, handling, testing, and release be articulated in advance of Mars sample return; second, that the protocols be reviewed regularly to update them to reflect the newest standards; and third, that international partners be involved in the articulation and review of the protocols.

Recommendation: *Detailed protocols for sample containment, handling, and testing, including criteria for release from a sample-receiving facility (SRF), should be clearly articulated in advance of Mars sample return. The protocols should be reviewed periodically as part of the ongoing SRF oversight process that will incorporate new laboratory findings and advances in analytical methods and containment technologies. International partners involved with the implementation of a Mars sample return mission should be a party to all necessary consultations, deliberations, and reviews.*

The NRC's 1997 Mars report recommended that: "Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it must . . . be sterilized" (p. 4). Subsequent NRC and NASA reports have made related, but in some cases conflicting, statements. Irrespective of these conflicts, there are critical issues concerning the selection of the aliquots for biohazard testing and the nature of the tests to be employed.

The discussion of advances in geobiology and biosignature detection in Chapter 4 raises the possibility that viable organisms might be preserved over a prolonged span of time within certain geological deposits. The discussion in Chapter 6 led the committee to conclude that the distribution of extant and fossil organisms and biomolecules in rocks, soils, and ices is heterogeneous at microscopic scales of observation, and this heterogeneity requires careful consideration because it complicates the selection of representative aliquots for biohazards testing.

Recommendation: *Future protocol guidelines should carefully consider the problems of sample heterogeneity in developing strategies for life detection analyses and biohazards testing in order to avoid sampling errors and false negatives.*

The limited amount of material likely to be returned from Mars demands that nondestructive means of analysis be employed to the maximum extent possible in sample characterization and biohazards testing.

Recommendation: *The best nondestructive methods must be identified for mapping the microscale spatial distributions of minerals, microstructures, and biologically important elements within returned martian samples.*

It is highly likely that many of the appropriate nondestructive methods will require the use of techniques that cannot feasibly be implemented within the confines of an SRF. Thus, a critical issue concerns the design of secondary containers for transporting samples to outside laboratory facilities where they can be analyzed (under containment) using advanced analytic techniques.

Recommendation: *Sample characterization in laboratories outside the primary sample-receiving facility will require the design of secondary containers for safely transporting samples and interfacing with a potentially wider variety of instruments.*

Technological Measures to Prevent the Inadvertent Release of Returned Samples

Planetary protection considerations require that martian materials be securely contained within a sample canister for their journey from Mars, through their collection and retrieval on Earth, and in subsequent transport and confinement in an SRF. With respect to the journey from Mars to an SRF, the NRC's 1997 Mars report concluded that the integrity of the seal of the sample canister should be verified and monitored during all phases of a Mars sample return mission. The present committee found this requirement to be overly prescriptive. Establishing the technical means to verify containment has proven to be a stumbling block in past mission studies. Elaborate steps must be taken to guarantee that the sample canister is sealed at every stage of its journey from Mars to an SRF. Resources might be better spent in simply improving containment (e.g., by using multiple seals) rather than designing elaborate means of monitoring. The first priority should be to ensure that the samples remain reliably contained until opened in an SRF. The means by which this result is achieved will best be determined by those designing the implementation of a Mars sample return mission.

Recommendation: *The canister(s) containing material returned from Mars should remain sealed during all mission phases (launch, cruise, re-entry, and landing) through transport to a sample-receiving facility where it (they) can be opened under strict containment.*

No facility currently exists that combines all of the characteristics required for an SRF. However, the committee found that there is a long, well-documented history of both the successful biocontainment of pathogenic and infectious organisms and a capability for maintaining the scientific integrity of extraterrestrial and planetary materials. Thus, the committee concluded that the requirement for handling and testing returned martian materials in a single facility combining both biocontainment and integrity-maintaining functions is both appropriate and technically feasible, albeit challenging.

The NRC's 1997 Mars report contained a four-part recommendation relating to various aspects of the establishment and operation of an SRF. The first part concerned the need for such a facility: "A research facility for receiving, containing, and processing returned samples should be established as soon as possible after serious planning for a Mars sample-return mission has begun" (p. 5). Although the present committee supports the intent of this recommendation, it emphasizes that the initiation of planning for an SRF must also include the initiation of planning for, and development of, the activities that will take place there.

Recommendation: *Because of the lengthy time needed for the complex development of a sample-receiving facility (SRF) and its associated biohazard-test protocol, instrumentation, and operations, planning for an SRF should be included in the earliest phases of the Mars sample return mission.*

The second part of the 1997 recommendation discussed the timescale for the establishment of an SRF: "At a minimum the facility should be operational at least 2 years prior to launch [of a Mars sample return mission]" (p. 5). The phrase "2 years prior to launch" is ambiguous because it could imply launch from Earth or launch from Mars. More specificity is needed as to the duration of the SRF's running-in period and the activities to be undertaken during that period. Recent experience with the design, construction, and/or commissioning of new BSL-4 facilities in the United States and overseas suggests that a 2-year running-in period is too optimistic. Facilities may become "operational" at BSL-2 or BSL-3 levels 2 years after completion but do not become fully operational as BSL-4 facilities for several additional years. Thus, it is essential to specify that an SRF be fully operational at least 2 years prior to the return of samples to Earth.

***Recommendation:** Construction and commissioning of a sample-receiving facility should be completed and fully operational at least 2 years prior to the return of samples to Earth, in order to allow ample time for integrated testing of the facility, the overall test protocol, and instrumentation well in advance of receiving returned martian materials.*

The third part of the 1997 recommendation concerned the roles and responsibilities of an SRF's staff: "The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization" (p. 5). The present committee concurs with this recommendation.

***Recommendation:** A sample-receiving facility should employ multidisciplinary teams of scientists to develop, validate, and perform a rigorous battery of tests that will be used to determine whether and when unsterilized materials returned from Mars may be approved for controlled distribution, or full release from containment.*

The final part of the NRC's 1997 recommendation concerning an SRF dealt with scientific oversight: "An advisory panel of scientists should be constituted with oversight responsibilities for the facility" (p. 5). The committee concurs with this recommendation, but in addition recommends including technical issues relating to an SRF within the oversight committee's terms of reference. The oversight committee's independence should also be specified.

***Recommendation:** An independent science and technical advisory committee should be constituted with oversight responsibilities for materials returned by a Mars sample return mission.*

Related Issues

Two additional important issues not specifically related to an SRF concern independent oversight of planetary protection policies and public engagement in activities related to Mars sample return.

The NRC's 1997 Mars report saw a need for high-level oversight of all planetary protection requirements associated with Mars sample return: "A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample-return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least 1 year prior to the establishment of the sample-receiving facility (i.e., at least 3 years prior to launch)" (pp. 5-6).

The committee does not believe that this recommendation is appropriate given the potential conflicts between planetary protection concerns and scientific or operational issues inherent in NASA's current advisory structure—i.e., with the Planetary Protection Subcommittee (PPS) reporting to the NASA Advisory Council (NAC) via the NAC's Science Committee. There is a critical need for the PPS, or its equivalent, and the NASA planetary protection officer to be formally situated within NASA in a way that will allow for the verification and certification of adherence to all planetary protection requirements at each stage of a Mars sample return mission, including launch, re-entry and landing, transport to an SRF, sample testing, and sample distribution. Clear lines of accountability and authority at the appropriate levels within NASA should be established for both the PPS (or an equivalent group) and the planetary protection officer, in order to maintain accountability and avoid any conflict of interest with science and mission efforts.

***Recommendation:** To ensure independent oversight throughout the lengthy and complex process of planning and implementing a Mars sample return mission, planetary protection policy and regulatory oversight for all aspects* *of sample return should be provided by both the Planetary Protection Subcommittee (or an equivalent group) and the NASA planetary protection officer, each having suitable authority and accountability at an appropriate administrative level within NASA.*

Finally, the NRC's 1997 Mars report recommended that: "Throughout any sample-return program, the public should be openly informed of plans, activities, results, and associated issues" (p. 6). The present committee concurs with this recommendation and believes that it is also important to explicitly extend the policy of openness to encompass both the sample-return mission and the construction, testing, and operation of an SRF.

Recommendation: *The public should be informed about all aspects of Mars sample return, beginning with the earliest stages of mission planning and continuing throughout construction, testing, and operation of a sample-receiving facility.*

NOTES

1. National Research Council, *Preventing the Forward Contamination of Mars,* The National Academies Press, Washington, D.C., 2006.
2. National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997.
3. National Research Council, *New Frontiers in the Solar System: An Integrated Exploration Strategy*, The National Academies Press, Washington, D.C., 2003, pp. 198-199.

1

Introduction

The past decade has seen a dramatic increase in knowledge of the history, geology, mineralogy, geochemistry, and physical properties of the martian surface. This increase in scientific knowledge has been enabled by a string of ambitious and highly successful orbital, lander, and rover missions over the past decade that continue to revolutionize understanding of the red planet. These missions and their major investigations are summarized in Table 1.1.

Recent successes in Mars exploration are attributable, in part, to the careful implementation of a well-coordinated, international effort initially articulated in NASA's 1995 report *An Exobiology Strategy for Mars Exploration.*[1] That document recommended a phased approach to Mars exploration that would alternate orbital and surface missions, with data acquired at each new opportunity advancing a discovery-driven program that would progressively focus and refine the selection of sites for surface exploration and, eventually, sample return. Spectral mapping from orbit has seen progressive increases in spatial resolution, over an expanded range of wavelengths, including probing of the subsurface by radar. Such observations have provided an increasingly detailed framework for identifying the best sites for landed missions, which have, in turn, provided ground-truth observations for interpreting the data from previous missions and refining the next generation of orbital investigations. This iterative strategy has allowed Mars exploration activities to become progressively more focused on a smaller number of high-priority sites for addressing fundamental questions about Mars, including the following:

- Past and present habitability,[2]
- The potential for life,
- Strategies for Mars sample return,
- Approaches to the containment and biohazard testing of martian samples, and
- The availability of resources and potential hazards for planning human missions.

IMPORTANCE OF MARS SAMPLE RETURN

A Mars sample return mission is acknowledged to be a major next step in the exploration of Mars.[3,4,5,6,7,8,9,10] Indeed, such a mission would provide essential support for answering many of the highest-priority scientific questions that have been identified by the international scientific community.[11] The NRC's 2003 solar system exploration decadal survey highlighted three areas where unambiguous answers to key science issues are unlikely without a sample-return mission:[12]

TABLE 1.1 Mars Spacecraft Missions and Investigations, 1965-2016

Operational at Mars	Spacecraft Name (Mission Type)	Agency	Science Investigations
Missions Operating Before Publication of *Mars Sample Return: Issues and Recommendations*[a]			
1965	Mariner 4 (Flyby)	NASA	Imaging system, cosmic dust detector, cosmic ray telescope, ionization chamber, magnetometer, trapped radiation detector, solar plasma probe, occultation experiment
1969	Mariner 6 (Flyby)	NASA	Imaging system, infrared spectrometer, ultraviolet spectrometer, infrared radiometer, celestial mechanics experiment, S-band occultation experiment
1969	Mariner 7 (Flyby)	NASA	Same as Mariner 6
1971-1972	Mariner 9 (Orbiter)	NASA	Imaging system, infrared spectrometer, ultraviolet spectrometer, infrared radiometer, celestial mechanics experiment, S-band occultation experiment
1976-1980 (Orbiter) 1976-1983 (Lander)	Viking 1 (Orbiter and Lander)	NASA	*Orbiter:* Imaging system, atmospheric water detector, infrared thermal mapper *Aeroshell:* Retarding potential analyzer, upper-atmosphere mass spectrometer *Lander:* Same as Viking 2
1976-1978 (Orbiter) 1976-1980 (Lander)	Viking 2 (Orbiter and Lander)	NASA	*Orbiter and Aeroshell:* Same as Viking 1 *Lander:* Imaging system, gas chromatograph mass spectrometer, seismometer, x-ray fluorescence, biological laboratory, weather instrument package, remote sampler arm
Missions Operating After Publication of *Mars Sample Return: Issues and Recommendations*[a]			
1997-2006	Mars Global Surveyor (Orbiter)	NASA	High-/medium-/low-resolution imager, thermal-emission spectrometer, laser altimeter, radio science experiment, magnetometer and electron reflectometer
1997	Mars Pathfinder and *Sojourner* (Lander and Microrover)	NASA	Panoramic imager, alpha proton x-ray spectrometer, atmospheric structure/meteorology package, magnetic properties of dust experiment
2001-Current	Mars Odyssey (Orbiter)	NASA	Thermal-emission imaging system, gamma ray spectrometer, neutron spectrometer, high-energy neutron detector, environmental radiation experiment
2003-Current	Mars Express (Orbiter)	European Space Agency	High-resolution stereo imager, subsurface and ionosphere sounding radar, infrared mineralogical mapping spectrometer, atmospheric Fourier spectrometer, ultraviolet/infrared atmospheric spectrometer, plasma and energetic atom analyser, radio science experiment
2003-Current	Mars Exploration Rovers *Spirit* and *Opportunity*	NASA	Panoramic stereo imager, thermal-emission imaging system, alpha particle x-ray spectrometer, Mössbauer spectrometer, microscopic imager, rock abrasion tool, magnetic properties of dust experiment
2005-Current	Mars Reconnaissance Orbiter	NASA	Visible/near-infrared imaging spectrometer, high-resolution imager, medium-resolution imager, low-resolution imager, infrared radiometer, shallow subsurface sounding radar
2008	Phoenix (Lander)	NASA	Panoramic stereo imager, soil electrochemistry and conductivity experiment (with atomic-force microscope), thermal and evolved gas analyzer, robotic arm, robotic-arm camera, lidar and meteorological package

TABLE 1.1 Continued

Operational at Mars	Spacecraft Name (Mission Type)	Agency	Science Investigations
Planned Future Missions			
To launch in 2011	Mars Science Laboratory (Rover)	NASA	Panoramic stereo imager, laser-induced breakdown spectrometer, alpha particle x-ray spectrometer, microscopic imager, x-ray diffraction/x-ray fluorescence instrument, gas chromatograph mass spectrometer, environmental radiation experiment, meteorological/environmental monitoring package, pulsed neutron generator/detector, descent imager
To launch in 2013	Mars Atmosphere and Volatiles Evolution (Orbiter)	NASA	Solar-wind electron and ion analyzers, suprathermal and thermal ion composition experiment, solar energetic particles, Langmuir probe and waves experiment, magnetometer, imaging ultraviolet spectrometer, neutral gas and ion mass spectrometer
To launch in 2016	ExoMars (Lander and Rover)	European Space Agency	*On rover:* Panoramic color camera, infrared mapper, ground-penetrating radar, close-up imager, Mössbauer spectrometer, laser Raman spectrometer, subsurface coring drill, multispectral microscopic imager for subsurface borehole studies, infrared microscope for characterization of drill cores and cuttings, x-ray diffractometer, gas chromatograph mass spectrometer and mass spectrometer for organic analysis, amino acid and chirality analyser *On lander:* Atmospheric radiation and electricity sensor, meteorological/environmental monitoring package, bistatic ground-penetrating radar, heat-flow sensor, radio science experiment, dust analyser, humidity sensor, magnetometer, seismometer, ultraviolet/visible spectrometer for atmospheric studies

[a] National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

- *The search for life.* As the most Earth-like planet in the solar system, Mars has historically provided a major focus for exploration to determine whether or not life exists, or has existed, elsewhere in the solar system. The Viking experience suggests that addressing questions of past or present martian life via in situ life-detection experiments is likely to lead to ambiguous results. Life detection can be addressed more thoroughly and systematically using returned samples collected from well-targeted locations, rather than with in situ robotic investigations.
- *Geochemical studies and age dating.* The history and evolution of Mars are encoded on a microscopic scale in the chemical and isotopic makeup of martian rocks. Study of a rock's constituent minerals, inclusions, and alteration products can reveal information on its age, its origins, the dates of thermal and aqueous alteration events, and a history of magmatic processes. Key to unlocking the rock record are careful sample selection and preparation.
- *Climate and coupled atmosphere-surface-interior processes.* Understanding the evolution of the martian climate over the past 4.5 billion years requires an understanding of the loss of atmospheric gases both to space and to surface reservoirs. Losses to the surface and to space leave characteristic isotopic signatures. Thus, compositional and isotopic analysis of surface minerals, weathering rinds, and sedimentary deposits can establish the climatic roles played by liquid water and processes such as weathering. Similar measurements of the volatiles released from near-surface minerals may provide fossils of past atmospheric and chemical conditions that allow past climate to be better understood. Again, careful sample selection and preparation are essential.

Although some progress toward addressing these three key areas can be achieved via in situ studies, returning samples to Earth is desirable for a number of reasons identified in reports issued by NASA and other major national and international space agencies. As noted in, for example, the recent report of the International Mars

Architecture for the Return of Samples (iMARS) Working Group,[13] returning samples to Earth is desirable for the following reasons:

- *Complex sample preparation.* Many high-priority science investigations will require sample preparation procedures that are too complex for in situ robotic missions (e.g., separation of minerals; extraction and concentration of trace elements and organic compounds using specialized solvents; chemical analysis using high-sensitivity instrumentation, or multiple analyses of samples; analyses at elevated temperatures; preparation and analysis of rock, or biological thin sections; high-magnification light and electron microscopy; and x-ray tomography).
- *Instrumentation not amenable to spacecraft application.* Certain kinds of instruments are simply too large, require too much power, or are otherwise unsuitable for flight missions.
- *Instrument diversity.* While the type and the diversity of instruments suitable for inclusion on a robotic Mars mission are limited, no such restriction applies to the instrumentation in terrestrial laboratories. Indeed, instruments and techniques unavailable at the time a mission is launched may be employed to full advantage for returned samples, allowing application of the most up-to-date, cutting-edge techniques.

In summary, although much has been accomplished through in situ robotic analysis and/or Earth-based laboratory studies of martian meteorites, returned samples from targeted locations on Mars will provide the best pathway for obtaining definitive answers to questions about the origin and evolution of Mars, including its climatic history, habitability, and life. Obviously, the most valuable returned samples will be those that come with detailed contextual information (e.g., precise spatial locations, geological settings, and so on) to directly link samples to past and present environmental frameworks.

With the advantages of Mars sample return comes an obligation to protect and preserve our home planet and all of its inhabitants against potential negative consequences of martian life forms returned to Earth. The purpose of this report is to provide an interim view of ongoing efforts to develop and implement plans for planetary protection for Mars sample return. In other words, it is the understanding of the Committee on the Review of Planetary Protection Requirements for Mars Sample Return Missions that its findings and recommendations will be applied at the tactical level by subsequent groups specifically charged with the development of implementable protocols for the collection, handling, transfer, quarantine, and release of Mars samples. That is the approach that was taken by NASA after its receipt of the National Research Council's (NRC's) 1997 report *Mars Sample Return: Issues and Recommendations*.[14] Indeed, the development of broad strategic guidelines by Space Studies Board committees and the subsequent development of tactical plans for their implementation by NASA committees is a general approach that has served the space-science community well for most of the past 50 years.

SAMPLE RETURN AND PLANETARY PROTECTION

In accordance with international treaty obligations,[15] NASA maintains a planetary protection policy to avoid biological contamination of other worlds, as well as to avoid the potential for harmful effects on Earth due to the return of extraterrestrial materials by spaceflight missions. NASA's implementation of the internationally accepted planetary protection guidelines—as promulgated by the Committee on Space Research (COSPAR) of the International Council for Science[16]—is based on advice and recommendations it receives from internal (e.g., the Planetary Protection Subcommittee of the NASA Advisory Council) and external (e.g., the NRC's Space Studies Board) advisory groups.

Planetary protection concerns can be divided into two components:

- Forward contamination, the inadvertent transfer of terrestrial organisms or biological contaminants to extraterrestrial bodies via spacecraft missions; and
- Back contamination, the transfer of putative biological materials and organisms to Earth via a sample return mission.

Although a 2006 NRC report dealt explicitly with forward-contamination issues relating to Mars missions,[17] it has been more than 10 years since back-contamination issues were examined.

Within COSPAR's guidelines, the planetary protection requirements levied on a particular spacecraft depend on the nature of its mission (e.g., flyby, orbiter, lander, or sample return) and the relevance of its destination to studies of chemical evolution and/or the origin of life. Each combination of mission type and destination is assigned a planetary protection category, with Category I being the least restrictive and Category V being the most restrictive. Each category has its own requirements for spacecraft cleanliness and bioload reduction before launch. Because Mars is of particular interest to astrobiology, it is subject to the strictest categories of planetary protection requirements. Missions such as flybys or orbiters that have no direct contact with the planet are designated as Category III, whereas landers, or probes that have direct contact at the surface, are designated as Category IV.

Category IV missions are subject to a variety of planetary protection requirements that depend on science objectives. Missions searching for extant martian life (e.g., the Viking landers) fall into Category IVb. Missions going to a place where liquid water is present, or where the presence of the spacecraft could cause liquid water to be present—so-called Special Regions—are Category IVc. Other missions to the surface (generally not investigating life; e.g., the Mars Exploration Rovers) fall into Category IVa.

All sample return missions, irrespective of their target, are designated as Category V. Target bodies like Mars, which are of direct interest to the search for extraterrestrial life, are further categorized as "restricted Earth return." This COSPAR categorization mandates that the following precautions be implemented:[18]

- "An absolute prohibition of destructive impact upon return" to Earth;
- The need for containment, during every phase of the return trip to Earth, of all returned hardware that directly contacts the targeted body, or any unsterilized materials from the body;
- A need to conduct timely analyses of any unsterilized samples collected and returned to Earth, under strict containment, and using the most sensitive techniques; and
- "The need for containment of any unsterilized samples collected and returned to Earth; if any sign of the existence of a non-terrestrial replicating entity is found, the returned sample must remain contained unless treated by an effective sterilizing procedure."

Of course, the COSPAR guidelines are not absolute and have evolved over time as new scientific information has become available. As already mentioned, the SSB last considered back-contamination issues associated with Mars sample return missions more than a decade ago. The resulting NRC report—*Mars Sample Return: Issues and Recommendations*[19]—provided specific recommendations for the handling of samples returned to Earth from Mars (Box 1.1). Those recommendations, combined with inputs from a series of workshops, resulted in the publication by NASA in 2002 of a draft protocol describing how martian samples should be studied to establish whether or not they pose a biological hazard to Earth.[20] However, at about the same time as the draft protocol was issued, a combination of budgetary and technical factors caused NASA to curtail its planning for a Mars sample return mission.

Renewed interest in Mars sample return by both NASA and the international space exploration community has urged a systematic review of the findings of the NRC's 1997 Mars report to update its recommendations based on current understanding of the biological potential of Mars, and in light of ongoing improvements in biological, chemical, and physical sample analysis capabilities and technologies. Although a detailed study is beyond the scope of the present activity, it is intended that the findings and recommendations that follow will provide useful interim advice for future NASA and international planning groups that will define a final protocol for handling and testing martian materials.

MARS EXPLORATION STRATEGY

The strategy for the astrobiological exploration of Mars responds to two major discoveries: first, that surface water environments were widespread on Mars early in the planet's history, and second, that potentially habitable

BOX 1.1
Recommendations from *Mars Sample Return: Issues and Recommendations* (1997)

Seven of the nine recommendations made in *Mars Sample Return*[1] concern the handling of samples returned from Mars.

- Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.
- Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized.
- The planetary protection measures adopted for the first Mars sample return missions should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent [oversight] body.
- A research facility for receiving, containing, and processing returned samples should be established as soon as possible after serious planning for a Mars sample return mission has begun. At a minimum, the facility should be operational at least 2 years prior to launch [of a Mars sample return mission]. The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization. An advisory panel of scientists should be constituted with oversight responsibilities for the facility.
- A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least 1 year prior to the establishment of the sample-receiving facility ([i.e.,] at least 3 years prior to launch).
- An administrative structure should be established within NASA to verify and certify adherence to planetary protection requirements at each critical stage of a sample-return mission, including launch, reentry, and sample distribution.
- Throughout any sample-return program, the public should be openly informed of plans, activities, results, and associated issues.

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[1] National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997.

environments may have existed at least locally in the near subsurface throughout the planet’s history. Accordingly, exploration follows two basic paths:[21,22]

- *Exopaleontology.* The search for ancient aqueous sedimentary deposits and habitable environments that may have preserved fossil biosignatures of past life; and
- *Exobiology.* The search for present habitable environments that could sustain extant life, with a focus on subsurface environments where liquid water could be present today, or the near-surface cryosphere, where remains of extant life forms may be preserved in ground ice or permafrost.

While surface water environments appear to have been widespread on Mars early in the planet's history,[23] liquid water has probably been present in the deep subsurface throughout the planet's history.[24,25] The oldest features identified on Mars, the cratered highlands, preserve a record of ancient habitable surface environments based on the widespread detection of sulfates and phyllosilicates from orbit by the OMEGA and CRISM imaging spectrometers on Mars Express and Mars Reconnaissance Orbiter, respectively.[26] Similarly, aqueously deposited carbonate minerals found in martian meteorite ALH 84001 suggest that potentially habitable environments were also present in the subsurface as early as 3.9 billion years ago.[27] (See also Chapter 2.) Evidence for out-floods of subsurface water during the Hesperian (i.e., the middle era of martian history) is preserved as large channels, such as Ares Vallis and Tui Vallis.[28] Similarly, the Eberswalde Crater provides evidence for standing bodies of water. Recent outflows of subsurface water have been suggested for volcanic sites, like Cerberus Rupes on the southern plains of Elysium (Figure 1.1),[29] and very recent gully features (Figure 1.2) carved by fluid seeps and springs have been identified at a large number of high-latitude sites on Mars.[30,31,32] These examples suggest that surface/near-surface liquid water environments have been present throughout the history of Mars and may still exist in the shallow subsurface.

While the discovery of potentially habitable environments on Mars (based on the inferred presence of water, bioessential elements, and energy sources) enhances the possibility that life could have originated there, it is understood that simply demonstrating the presence of factors considered necessary for life on Mars is inadequate assurance that life actually originated there. This is why we explore!

Although there are important planetary protection implications associated with returning samples from any location on Mars, the greatest risk will be incurred by missions that return samples from so-called Special Regions,[33] where habitable conditions may sustain viable organisms.

The Viking lander mission established that present surface conditions on Mars are unfavorable for life as we understand it,[34] and although favorable conditions may exist in the deep subsurface today, robotic technologies for deep drilling on Mars remain a distant prospect. Thus, the current path for the surface exploration of Mars places an emphasis on the search for fossil biosignatures preserved in ancient, water-formed sedimentary deposits.[35]

The "build on your successes" approach followed during the past decade of Mars exploration has been enabled by the Mars community's concerted effort to rapidly disseminate new data. As a result, NASA's Mars Exploration Program has remained highly responsive to new discoveries, feeding new results into planning efforts for future missions. For example, discoveries of Mars Global Surveyor directly supported NASA and community-based efforts to prioritize and select landing sites for the highly successful Mars Exploration Rover mission,[36,37] by providing planning insights up until 6 months before launch. Discoveries of the Mars Odyssey and Mars Reconnaissance Orbiter (MRO) missions also played a major role in the selection of the landing site for Phoenix.[38] The combined efforts of Odyssey, MRO, and the European Space Agency's Mars Express missions are likewise providing new high-resolution data for selecting the best landing sites for the Mars Science Laboratory rover, currently scheduled for launch in 2011.

Given the past successes of the phased strategy for exploration outlined above, it seems clear that data from current orbital and landed missions will continue to play crucial roles in the targeting of a site or sites for future Mars sample return(s).

REPORT ORGANIZATION

Since the purpose of this document is to revise, update, and replace the NRC's 1997 report *Mars Sample Return: Issues and Recommendations*, it is most logical to organize it around the basic question, What has changed since the release of the 1997 report? Changes that have an impact on planetary protection requirements can be divided into two categories: changes in scientific understanding and changes in the technical and/or policy environment. Changes is scientific understanding have been organized as follows:

- *New insights on the roles played by surface and subsurface water throughout martian history and the potential for habitable environments on Mars*. Chapter 2 provides a brief review of major discoveries in Mars exploration over the past decade that have shaped current understanding of the potential for past and present habitability. At the heart of this exploration effort has been a guiding principle, "follow the water," based on the rationale that water in its liquid state provides a useful proxy for habitability. Pursuit of this principle for Mars has sustained remarkable successes, with new evidence for liquid water, both at the surface in the past and in the shallow subsurface of Mars, throughout much of the planet's history. The discovery of a diverse subsurface biosphere on Earth has opened up possibilities for habitable zones of liquid water in the martian subsurface, where chemotrophic microbial life forms may survive by exploiting simple chemical sources of energy, such as carbon dioxide, hydrogen, or methane. This understanding has driven the development of radar instruments to explore for a subsurface hydrosphere on Mars from orbit (e.g., Mars Advanced Radar for Subsurface and Ionosphere Sounding (MARSIS) and Shallow Radar (SHARAD) experiments onboard Mars Express and MRO, respectively). Chapter 2 also examines progress toward understanding the potential for past habitability on Mars, based on studies of martian meteorites. Over the past decade, advances in laboratory instrumentation and new analytical capabilities, applied to studies of martian meteorites, have provided new insights into the nature of past martian environments and the role that water has played in the alteration of crustal rocks on Mars.

- *Advances in microbial ecology that illuminate the limits of adaptability of life on Earth.* Chapter 3 reviews recent discoveries that have shaped a new understanding of the basic requirements for living systems on Earth, including an expanded awareness of the environmental extremes occupied by microbial life and the diverse array of energy sources life utilizes. Again, one of the most significant discoveries is the ability of deep-subsurface life to survive on simple forms of chemical energy, which on Earth supports a vast subsurface biosphere.[39,40] Such discoveries require a focusing of the "follow the water" strategy—that is, the institution of a strategy encompassing a nested set of requirements, with the availability of past/present water as the first step. Subsequent steps would be prioritized according to the inferred availability of the elemental building blocks and energy sources required for life. This focusing of the search strategy is evident in the enhanced payload capabilities of the Phoenix lander, which analyzed the chemistry of frozen regolith, and the Mars Science Laboratory and ExoMars missions, which are scheduled to deliver sophisticated biogeochemistry and organic chemistry experiments, respectively, to sites where orbital data provide strong evidence for past water. These efforts represent a renewal of Viking's initial search in 1976 for organic matter preserved in rocks and ices on Mars, and they provide logical next steps toward in situ life detection experiments that should precede Mars sample return.
- *New understanding of the physical and chemical mechanisms by which evidence of life might be preserved on Mars and how that life might be detected in martian samples.* Chapter 4 discusses developments in the field of geomicrobiology that have significantly advanced understanding of the varied roles that microorganisms play in sedimentary processes and have helped define new approaches for the astrobiological exploration of Mars. It provides a look at the role that terrestrial analog studies have played in advancing the understanding of habitability and in refining strategies for Mars exploration. In particular, studies of environmental molecular biology and processes of microbial fossilization in Mars analog environments on Earth have helped to refine approaches to in situ and laboratory-based biosignature detection, while studies of the fossilized remains in ancient Precambrian sediments have provided insights into the nature of preservational biases and the effects of post-burial alteration on the long-term retention of fossil biosignatures under different post-burial histories. Such studies continue to lay important groundwork for future in situ missions and Mars sample return.
- *New understanding of pathogenesis and the nature of biological epidemics.* Chapter 5 briefly reviews how new developments in the biomedical community have affected understanding of the possibility that putative martian organisms may be pathogenic.
- *Additional insights as to the possibility that viable martian organisms might be transported to Earth by meteorites.* Chapter 5 also discusses the natural interchange of materials between planets and the possibility that hypothetical martian organisms might survive ejection from Mars and transport to Earth.

The changes in the technical and/or policy environment can be organized as follows:

- *A significant expansion of the size of the Mars exploration community and broadening of the scope of mission activities by both traditional and new space powers.* Chapter 2 explores how a decade's worth of highly successful Mars missions has resulted in significant growth in the size of the Mars exploration community inside

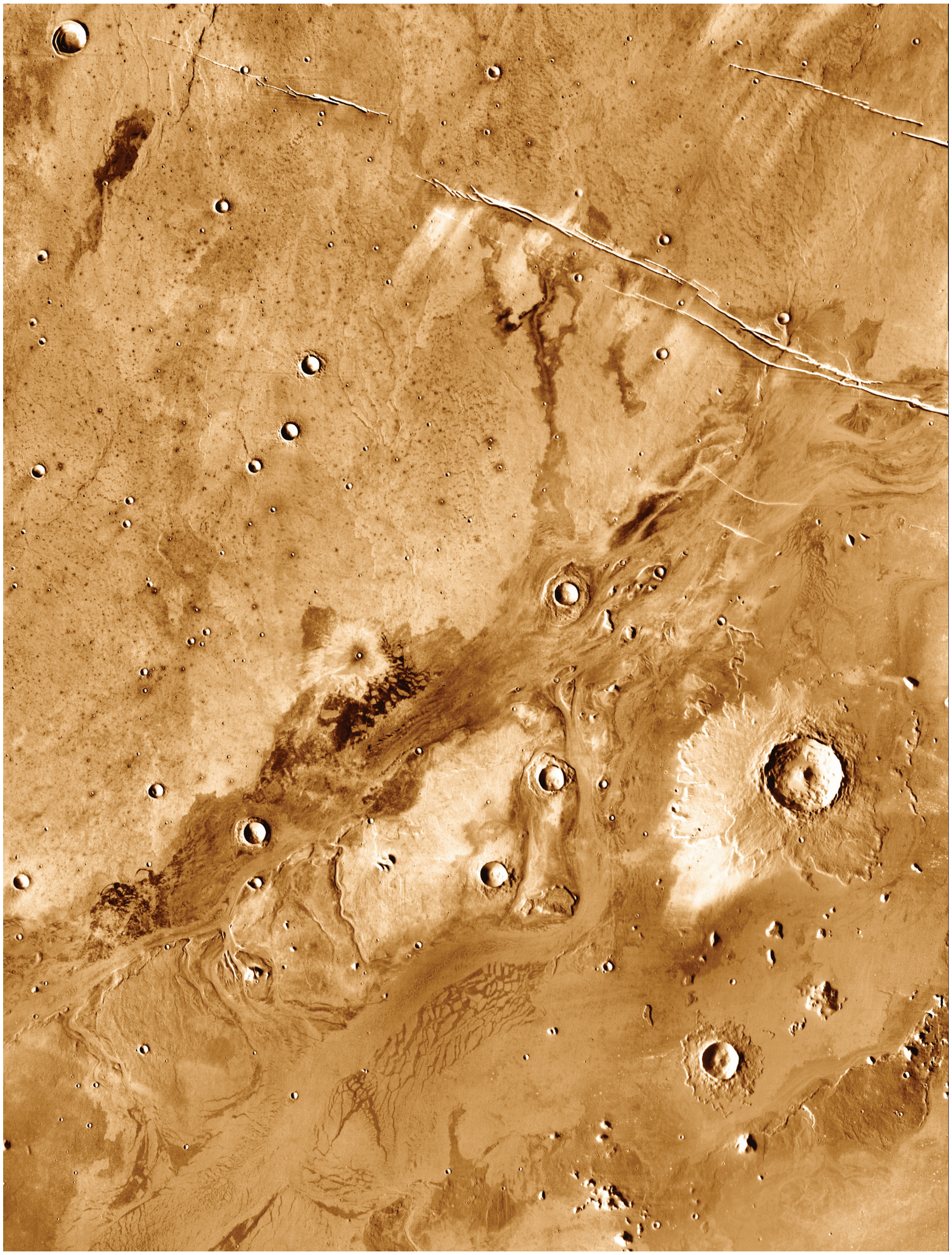

FIGURE 1.1 A colored image mosaic taken by the Thermal Emission Imaging System onboard the Odyssey spacecraft. This 240-km by 320-km image covers portions of the Cerberus Fossae (volcanic fissures) and the upper reaches of the Athabasca Valles channel system believed to have been formed by repeated outbursts of subsurface water, perhaps within the past 2 million years. Note the streamlining of deposits around impact craters located at the heads of some streamlined islands. Athabasca Valles lies ~1,000 kilometers southeast of the large martian volcano, Elysium Mons. SOURCE: Courtesy of NASA/Jet Propulsion Laboratory and Arizona State University.

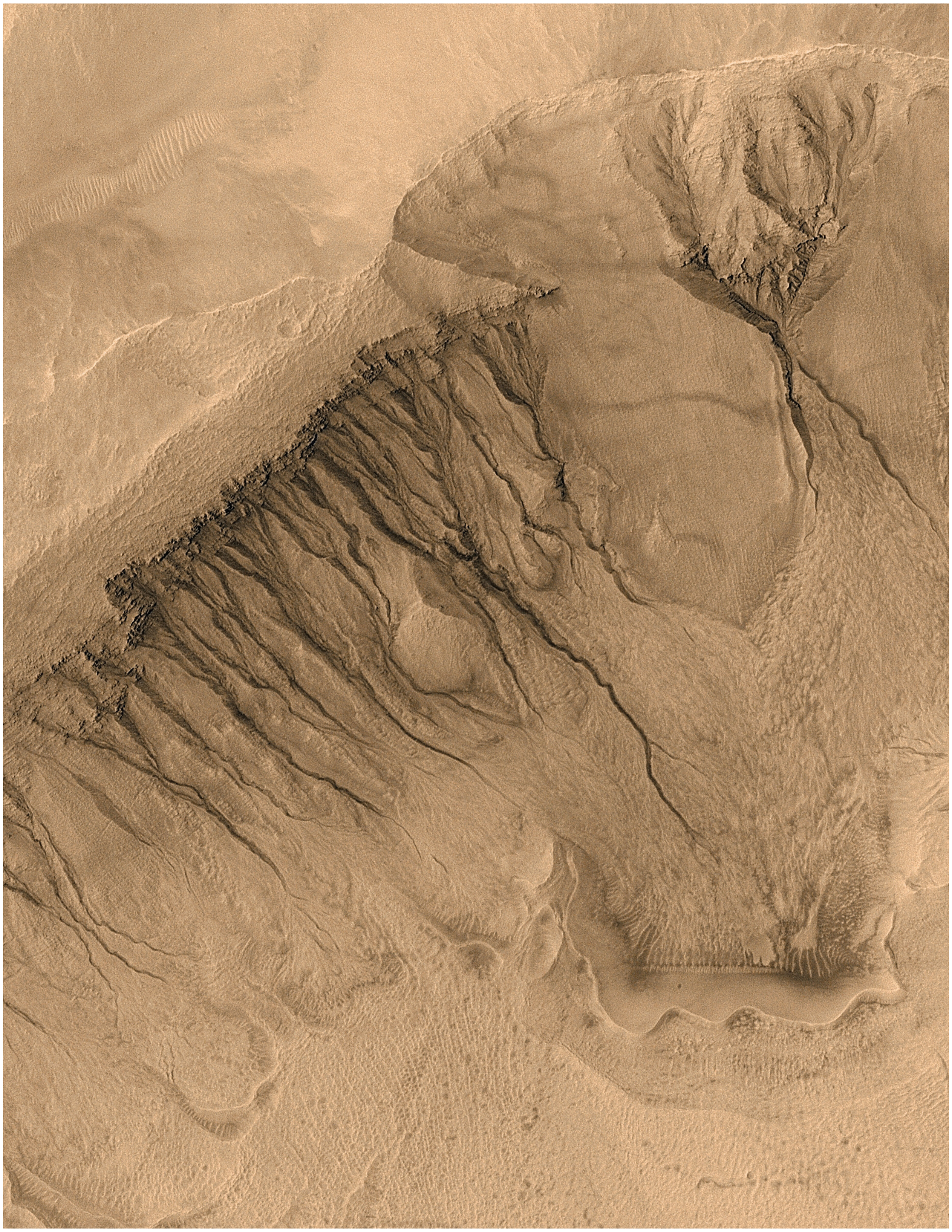

FIGURE 1.2 Mars Orbiter Camera image (E11-04033) showing the north wall of a small (7-km-diameter) impact crater (39.1°S, 166.1°W) located within Newton Crater. The image shows numerous small gullies hypothesized to have been formed by water and sediment-laden debris flows, which formed lobe-shaped deposits at the base of the crater wall. The image shows an area approximately 3 km across. SOURCE: Courtesy of NASA/Jet Propulsion Laboratory and Malin Space Science Systems.

and outside the United States. Other factors of significance include the democratization of priority-setting exercises and the internationalization of mission activities. The combined effect of all these developments has been a significant acceleration in the pace of acquisition of new information about Mars.

- *Greater societal awareness of the potential for technical activities to cause harmful changes in the global environment.* Chapter 5 briefly examines the potential for large-scale negative effects resulting from the inadvertent release of pristine martian materials and possible extraterrestrial life forms into Earth's environments. It also provides an update of the concept of panspermia and the potential for natural transfers of putative martian organisms to Earth by meteorites. Advances in modeling have helped to clarify the potential for exchanges of crustal materials between Earth and Mars by impact ejection. In addition, studies of the prolonged survival of microorganisms under extreme conditions have also shed new light on the potential for life forms to survive impact ejection, interplanetary transport, and landing on another planetary surface.
- *The de facto internationalization of a Mars sample return mission and subsequent sample-handling, sample-processing, sample-analysis, and sample-archiving policies.* Chapter 6 raises complications that might arise for Mars sample return given that the execution of such a mission is likely to be beyond the resources of NASA or any other single agency. An international Mars sample return mission might suffer if differences in national policies and legal frameworks significantly complicate issues relating to sample quarantine policies and biohazard certification.
- *The drafting and publication by NASA, with the assistance of international partners, of initial Mars sample-handling and biohazard-testing protocols based on the recommendations in the NRC's 1997 Mars Sample Return report.* Chapter 6 compares and contrasts the policies for biohazard testing and criteria for releasing samples from containment included in NASA's draft protocols and other recent reports.
- *The development of nondestructive methods of analysis that can be used to map the microscale spatial distribution of minerals and biological elements in samples.* Chapter 6 also discusses an issue arising from biohazard tests that will require the selection of small, representative subsamples from larger samples of martian materials. Advances in geomicrobiology (Chapter 4) have indicated that the distribution of biosignatures in rocks, soils, and ices is typically highly heterogeneous at the microscopic scale. This distributed heterogeneity raises concerns about how best to obtain representative samples that will yield reliable results during testing for potential biohazards. The application of new analytical techniques that can be used to map the microscale spatial distribution of minerals and biological elements in samples might provide a solution.
- *The proliferation of biocontainment facilities driven by biosecurity concerns and associated changes in public policy and in the public acceptance of such facilities.* Chapter 7 examines the unique characteristics of a Mars sample-receiving facility and discusses previous recommendations for such containment facilities, including broader issues related to Mars sample return program oversight and public communication.
- *Lessons learned about the practical and logistical aspects of Mars sample return from experience with the Genesis and Stardust missions as well as experience gained from the planning for and commissioning of new biocontainment facilities.* Chapter 7 summarizes the lessons learned from recent sample return missions, particularly as they relate to the landing, transport, and testing of extraterrestrial materials on Earth.

Each chapter ends with conclusions and/or recommendations that suggest actions that should be considered during future science and technology planning efforts for Mars sample return.

NOTES

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2

The Potential for Past or Present Habitable Environments on Mars

Evaluation of the potential for living entities to be included in samples returned from Mars requires a careful consideration of the nature of past and present habitable conditions on the red planet, both at the surface and in the subsurface. Thus, future investigations that could help to reduce uncertainty in assessments of the potential for living entities to be present in returned martian samples include detailed site investigations prior to Mars sample return to define geological and environmental contexts and assess the potential for past or present habitable conditions.

An important focus of the current surface robotic program for Mars is the discovery of past or present habitable environments as a context for selecting sites for future life detection experiments and Mars sample return. It is important to continue such targeted site investigations in preparation for Mars sample return to provide insight into the kinds of samples that will be returned. It seems logical that to address the question of extant martian life, Mars sample return will be targeted to a Special Region[1] where habitable conditions exist today, or may have existed in the recent past. In contrast, to explore for fossil biosignatures in ancient sediments, Mars sample return should be targeted to sites that are likely to have had habitable conditions in the past but that might not support extant life today. In either case, a detailed characterization of the site prior to Mars sample return would enhance understanding of the potential for samples to contain life, whether extant or fossil, and would provide essential context for interpreting sample data, if or when samples are collected, returned, and analyzed from that site.

When considering the advances in understanding of Mars that have occurred in the past decade, it is important not to forget that the scientific environment for martian studies has undergone considerable change. A decade's worth of successful missions has caused significant growth in the size of the Mars exploration community and the scope of mission activities. The U.S. scientific community has played a highly active role in the definition of future science goals and mission plans through the activities of, for example, the Mars Exploration Program Analysis Group and the initiation of a solar system exploration decadal survey process. The Mars exploration community is now thoroughly international with, for example, the development of an ambitious Mars exploration program by the European Space Agency, a resurgence of Russian interest, and the initiation of exploration activities by new space powers such as China and India. The combined effect of all these trends has been a significant acceleration in the pace of acquisition of new information about the origin and evolution of the martian environment.

FOLLOWING THE WATER ON MARS

Water is an essential requirement for terrestrial life. The history of water in all its forms is central to an understanding of the geologic and climatic history of Mars and for assessing the potential for past or present habitable environments on Mars. Water is also an essential resource that will be needed to sustain future human exploration of Mars.

Discoveries over the past decade have revealed water to be abundant on Mars today, mostly in the form of surface and subsurface ice and, to a lesser extent, as ephemeral water films in soils, or as atmospheric water vapor. The Gamma Ray Spectrometer investigation onboard the Mars Odyssey orbiter confirmed high concentrations of water ice buried just a few centimeters below the surface in both hemispheres poleward of ~60° latitude.[2,3] The MARSIS radar experiment on Mars Express has shown that in many places this buried, ice-rich layer may be on the order of a kilometer thick.[4] One of the major scientific results of the Phoenix mission was the in situ confirmation of this high-latitude, ground ice reservoir—sitting literally just beneath the spacecraft (Figure 2.1).[5]

FIGURE 2.1 Image acquired by the Surface Stereo Imager on NASA's Phoenix lander. The view is of a 22-centimeter-wide trench excavated by the lander's robotic arm on Sol 18. The white material exposed in the floor of the trench is interpreted to be water ice. SOURCE: Courtesy of NASA/Jet Propulsion Laboratory-California Institute of Technology/University of Arizona/Texas A&M University.

Investigations made of the residual north polar cap using the Mars Reconnaissance Orbiter's (MRO's) SHARAD radar have shown it to be composed almost entirely of pure water ice.[6] It has been suggested that sublimation of some of this water ice during the summertime significantly increases the global abundance of water vapor in the atmosphere.[7,8]

In addition, investigations from nearly all of the active Mars missions during the past decade have provided complementary evidence for the presence of minerals deposited under past aqueous conditions, some of which still contain chemically bound water. For example, observations with Mars Global Surveyor's Thermal Emission Spectrometer led to the discovery of coarse-grained, crystalline hematite (typically formed in water) at Sinus Meridiani, the landing site for the Mars Exploration Rover *Opportunity*.[9,10] In combination, Mars Odyssey, the Mars Exploration Rovers, Mars Express OMEGA, and MRO CRISM infrared spectrometer have discovered significant deposits of hydrated ferric oxides,[11] hydrated sulfate minerals (Figure 2.2),[12,13,14,15,16] hydrated phyllosilicate (clay) minerals,[17,18,19] amorphous silica deposits,[20] and putative chloride salt deposits.[21] These mineralogical discover-

FIGURE 2.2 Color image of bedrock outcrop called El Capitan, a finely layered, sulfate-rich deposit exposed in Eagle Crater, landing site of the Mars Exploration Rover *Opportunity*. The bluish-colored spherical grains are concretions 1 to 2 millimeters in diameter that have been cemented by an iron-oxide (hematite). Both the sulfates and the hematite were deposited from water. SOURCE: Courtesy of NASA/Jet Propulsion Laboratory and Cornell University.

ies indicate that a broad range of potentially habitable liquid water environments have existed on Mars over the planet's long history.

In addition to mineralogical discoveries made over the past decade of Mars exploration, there have also been important geological discoveries that have further enhanced the potential for the presence and duration of past aqueous environments. This evidence includes the discovery of:

- Small, morphologically "fresh" gullies along the inner walls of many equatorial and midlatitude impact craters;[22,23,24]
- Valley network and alluvial fan-like features that suggest past rainfall and surface runoff;[25,26]
- What appear to be river deltas within closed sedimentary impact basins that might once have held crater lakes;[27,28]
- Widespread evidence (seen in high-spatial-resolution images) for rhythmically layered sedimentary rocks across much of the planet;[29] and
- Fine laminations and shallow trough cross-bedding interpreted to have formed by aqueous sedimentation in sulfate-rich outcrops imaged by the *Opportunity* rover at Meridiani Planum.[30,31]

MARTIAN METHANE

Another significant set of results from Mars that postdates the release of the NRC's 1997 report *Mars Sample Return: Issues and Recommendations*[32] concerns the spectroscopic detection of methane in the planet's atmosphere both by ground-based telescopes[33,34] and by the Mars Express spacecraft.[35] Although the spacecraft results are still somewhat controversial, the most recent and definitive ground-based measurements point to the presence of methane in the planet's atmosphere at mixing ratios that vary between <3 parts per billion (by volume) and 60 parts per billion.[36] Intriguing aspects of these latest findings include the following:

- At some times, the methane mixing ratio correlates with the mixing ratio of water in the martian atmosphere. But at other times it does not.
- The methane appears to be localized over certain geographic features including Terra Sabae, Nili Fossae, and the southeastern quadrant of Syrtis Major.
- The measured lifetime of the methane in the martian atmosphere is <4 years. This is significantly less than the ~350 years expected if the principal mechanism of loss is photodissociation.

Although the origin of the methane has not yet been determined, possible sources include volcanic activity, chemical reactions between water and iron-bearing minerals in hydrothermal systems, and biological activity. Confirmation of the methane observations will be an important goal for future Mars orbiters and landers.

IMPLICATIONS FOR HABITABILITY

Discoveries made during the past decade of Mars exploration hold profound implications for the past and present habitability of Mars and the potential that returned samples from Mars might include living entities, or their fossilized remains. For example, during the past decade, the presence of ground ice has gone from a hypothetical construct[37] to an actual, measured reality—at least in the near surface.

During this same period, there have been significant advances in understanding of the environmental limits of habitability on our own planet (see Chapter 3). Conditions for the origin and persistence of life are at present unknown. However, it is presumed that basic requirements for life include the presence of liquid water. Conservative estimates constrain life's propagation to $T > -25°C$ and a thermodynamic water activity[38] of $a_w > 0.5$,[39,40] although there is some limited evidence for the maintenance of metabolic activity at $a_w = 0.3$.[41] Surface environments in equilibrium with the current atmosphere of Mars do not appear to meet these basic requirements for habitability. And although liquid water could exist transiently as thin films at or near the surface, such circumstances are likely to be both rare and short-lived. However, habitable zones of liquid water could be present deeper in the subsurface, where pressures and temperatures provide conditions that favor the stability of liquid water. Some have modeled the presence of a martian deep hydrosphere,[42] and others have suggested that if present, a martian subsurface hydrosphere should be dominated by salt-rich brines.[43]

Dissolved salts lower the freezing point of water, allowing it to remain liquid at temperatures below –25°C. However, the activity of water in concentrated brines is low, which limits the potential for life. The effects of salinity and water activity on the habitability of cold, hypersaline environments on Earth are still poorly constrained,[44] and the salinity of a putative martian subsurface hydrosphere is unknown. An improved understanding of both of these research areas will be crucial for refining exploration for habitable zones on Mars and in defining Special Regions as potential targets for Mars sample return.

INSIGHTS GAINED FROM THE STUDY OF MARTIAN METEORITES

Understanding of past and present environmental conditions on Mars has also been advanced through studies of martian meteorites that have been found on Earth. The SNC meteorites (named for the Shergotty, Nakhla, and Chassigny meteorites that are representative of this class) are believed to have been ejected from Mars into heliocentric orbits by large impacts and subsequently captured by Earth.[45] The evidence for a martian origin is compelling, and a broad consensus now exists in the scientific community that this class of meteorites indeed came from Mars. To date, more than 30 martian meteorites have been found on Earth. This number has continued to increase each year through sustained, international discovery efforts supported by NASA, the National Science Foundation, and other agencies.

During the past decade, significant advances in measurement capabilities have enabled the identification of accessory mineral assemblages in martian meteorites. This progress has yielded new insights into the nature of martian crustal environments and the role that water has played in the alteration of rocks and soils. Specifically, the identification of accessory mineral phases in SNC meteorites and precise measurements of isotopes for hydrogen, carbon, oxygen, and sulfur for aqueous phases have provided important information about interactions in the atmosphere-regolith-water system. These observations have supported the development of models to specify the mechanisms by which surface sulfur is admixed into martian subsurface reservoirs. Results hold important implications for the history of water on Mars and the nature of past habitable environments and life. Similar measurements for other (nonmartian) meteorites have also extended the discussion of habitability in the solar system by showing that hydrothermal conditions once existed on some asteroidal bodies.[46]

While water vapor was detected in Mars's atmosphere via telescopic measurements and water-ice was unambiguously detected at the martian north pole by Viking,[47,48] the first direct measurement of the isotopic composition of water in a martian sample was obtained by the stepwise thermal decomposition and release of water from the SNC meteorites Nakhla and Chassigny.[49] That study revealed several important features, including the following:

- Water on Mars is not in equilibrium with the host rock, presumably due to the absence of plate-tectonic recycling of the crust;
- The composition of water in the martian regolith has evolved over time through groundwater circulation and precipitation of secondary mineral phases; and
- The carbonates observed in SNC meteorites were formed on Mars and precipitated by circulating fluids that constitute the subsurface water reservoir.

Other measurements of the isotopic systems for hydrogen and carbon have added information about the precipitation of secondary alteration minerals, further refining the understanding of fluid compositions and their evolution.[50,51,52,53]

The carbon dioxide in the martian atmosphere possesses a highly specific isotopic signature, owing to its interaction with electronically excited atomic oxygen ($O\ {}^1D$), a product of the ultraviolet photolysis of ozone. The first direct evidence for interactions between the martian atmosphere and surface water reservoirs was provided by

Farquhar and colleagues.[54,55] These studies demonstrated that SNC carbonates show a mass-independent, oxygen-isotope anomaly that cannot be completely ascribed to equilibrium exchange between martian surficial water and carbon dioxide. Rather, the isotopic signature of oxygen in carbonates derives from water in the surface reservoir of Mars that has interacted with isotopically anomalous atmospheric carbon dioxide (Figure 2.3).

The development of secondary ion mass spectrometry (SIMS) has provided a means for measuring the isotopic composition of individual crystals within samples. The enhanced resolution possible with this method has significantly advanced the ability of researchers to discriminate between martian hydrological reservoirs and processes. For example, using this approach, Valley and colleagues observed the same isotopically anomalous oxygen isotopic signature in martian SNC carbonates observed previously by conventional bulk analysis methods.[56,57] These discoveries have provided additional support for the argument that the SNC carbonates record exchanges between the surface regolith and water reservoirs, through subsurface groundwater transport. This model also provides a mechanism for the active transport of oxidation products from surface to subsurface reservoirs, via circulating groundwater. This opens possibilities for redox-based energy sources essential for life, such as the oxidation of ferrous iron and reduced sulfur compounds.

Other details have emerged regarding the martian atmosphere-regolith system with the measurement of all three oxygen isotopes in SNC sulfates.[58] The measurement of the isotopic partitioning between silicates, carbonates, and sulfates in SNC meteorites has so far provided the best record of martian water-mediated geochemical

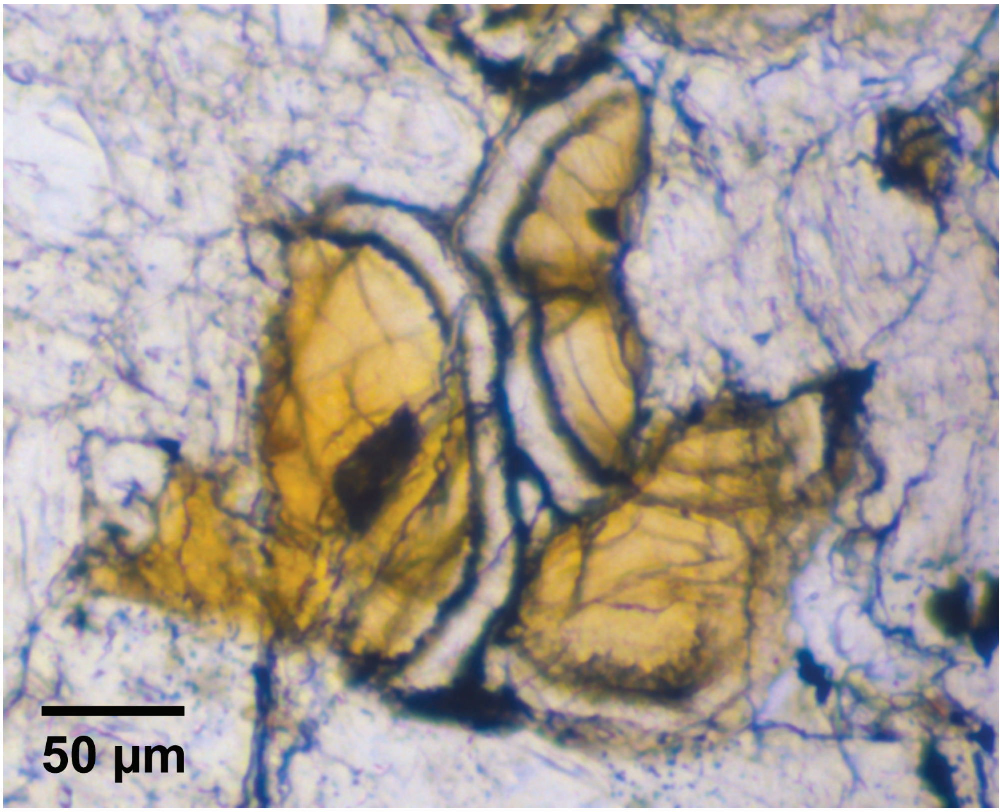

FIGURE 2.3 Photomicrograph of a thin section of the martian meteorite ALH 84001 showing white-rimmed orange blebs of Fe-rich carbonate (siderite), a secondary mineral deposited from water. The view is about 0.5 millimeters across. SOURCE: Courtesy of Allan Treiman and the Lunar and Planetary Institute.

processes. Laboratory investigations of the sulfur oxidative process and the concomitant isotopic partitioning[59] suggest that the predominant oxidants were hydrogen peroxide and/or ozone. This indicates that the primary oxidants had sufficient electro-negativity to impart their isotopic composition to any secondary minerals formed. If so, then subsurface water circulation would have been restricted, with water/rock ratios remaining low.

Although the significance of these observations for the origin and persistence of putative subsurface martian life forms is still unclear, the isotopic record of secondary aqueous minerals (carbonates and sulfates) in the SNC meteorites provides a direct record of hydrological processes of great importance for assessing the long-term habitability of the martian subsurface.

In summary, the application of new high-resolution isotopic methods to the study of martian meteorites suggests the following:

- Liquid water has existed in the martian subsurface over prolonged periods of geological time;
- Active exchanges between surface and subsurface water reservoirs maintained by groundwater circulation provided a means for the active transport of oxidants needed to maintain subsurface redox gradients; and
- The abundance of water was sufficient for authigenic mineral precipitation, but relative to the host rock, water volumes have remained low.

CONCLUSIONS AND RECOMMENDATIONS

The assessment of martian habitability made in the NRC's 1997 report *Mars Sample Return: Issues and Recommendations* led to its recommendation that: "Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized" (p. 3). The present committee found that the knowledge gained from both orbital and landed missions conducted over the past decade, combined with findings from studies of martian meteorites, has enhanced the prospect that habitable environments were once widespread over the surface of Mars. In addition, the potential for modern habitable environments, both as transient surface environments and as stable habitats in the deep subsurface, is much better understood. This understanding has, in turn, enhanced the possibility that living entities could be present in samples returned from Mars. Therefore, the committee concurs with and expands on the 1997 recommendation that no uncontained martian materials should be returned to Earth unless sterilized.

Recommendation: *Based on current knowledge of past and present habitability of Mars, NASA should continue to maintain a strong and conservative program of planetary protection for Mars sample return. That is, samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.*

The committee found that uncertainties in the current assessment of martian habitability and the potential for the inclusion of living entities in samples returned from Mars might be reduced by continuing research in the following areas:

- A vigorous program of remote-sensing and in situ exploration of Mars with the goal of answering questions relating to martian habitability, including those concerned with the presence of water in surface and subsurface environments through time, the distribution of biogenic elements, and the availability of redox-based energy sources (e.g., those based on the oxidation of ferrous iron and reduced sulfur compounds); and
- Continued studies of martian meteorites to help refine understanding of the history of interactions of Mars's rock-water-atmosphere system throughout the planet's history.

NOTES

1. Special Regions are places where liquid water is present, or where the presence of a spacecraft could cause liquid water to be present.

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3

Advances in Microbial Ecology

Studies that can further reduce uncertainties in estimates of the potential for living entities to be included in martian samples also encompass those that seek to understand the ecological diversity and environmental extremes of terrestrial life. Understanding the range of environmental conditions to which terrestrial life has adapted has directly shaped current views of martian habitability and of the potential for samples returned from Mars to contain evidence of life. A substantial and growing body of evidence shows that life not only is present, but also frequently thrives under extreme environmental conditions.[1] The known limits of life now extend from superheated deep ocean hydrothermal vents, to ice-brine environments of polar regions, from highly acidic waters of mine drainage systems, to ephemeral, hypersaline alkaline and acidic lakes, from sunlit surface environments, to perpetually dark subsurface aquifers located thousands of meters underground. Even more remarkably, biologists continue to discover new biological entities, such as the giant *Acanthamoeba polyphaga* mimivirus;[2] virophages that prey on other viruses;[3] and novel single-species ecosystems, such as the deep subsurface bedrock fractures inhabited solely by the chemoautotrophic *Candidatus Desulforudis audaxviator*.[4]

EXAMPLES OF LIFE IN EXTREME ENVIRONMENTS ON EARTH

The broad range of environmental extremes capable of sustaining terrestrial life is surpassed only by the physiological, metabolic, and phylogenetic diversity of their extremophile inhabitants (Table 3.1). These unique life forms include not only eukaryotes, bacteria, and archaea, but also viruses.[5,6,7] Moreover, the discovery of independent viral growth *outside* a host cell, under acidic hyperthermophilic conditions, indicates that viruses are more complex biologically than the scientific community has previously assumed.[8] Although geological extremophiles have not yet been shown to pose significant biological risks to humans given their inability to cause disease or environmental contamination, discoveries of new organisms and ecological interactions, such as those discussed above, do influence perceptions of the potential for martian life.

In many extreme ecosystems, chemoautotrophs are the sole primary producers of organic matter;[9] this is especially so in perpetually dark environments of the deep seafloor or subsurface crust, where life fundamentally depends on inorganic forms of energy and carbon sources (e.g., hydrogen and carbon dioxide).[10] By some estimates the subsurface biosphere accounts for as much as 85 percent of the microbial biomass, and up to 30 percent of the total living biomass, on Earth.[11] These observations suggest that, given the hostile conditions at the martian surface, the potential for martian life is likely to be much greater in subsurface environments than at the surface of the planet.

TABLE 3.1 Environmental Limits for the Growth of Extremophilic Organisms

Parameter	Classification	Definition	Example
Temperature	Hyperthermophile	Growth >80ºC	Archaeal strain 121;[a] 121ºC *Methanopyrus kandleri*[b]
	Thermophile	Growth 60º to 80ºC	*Pyrolobus fumarii;* ~116ºC
	Psychrophile	Growth <15ºC	*Synechococcus lividis;* ~73ºC
		Active at −18ºC	*Psychrobacter* Himalayan midge[c]
pH	Acidophile	Low pH (<5)	*Ferroplasma acidarmanus;*[d] pH 0
	Alkaliphile	High pH (>9)	*Alkaliphilus transvaalensis,*[e] pH 12.5 *Natronobacterium;* pH 10.5
Salinity	Halophile	2 to 5 molar NaCl	Halobacteriaceae
Oxygen tension	Aerobe	Requires O_2	Bacteria, archaea
	Microaerophile	Tolerates some O_2	Neutral pH Fe^{2+}-oxidizing bacteria
	Anaerobe	Not tolerant of O_2	Methanogens, SO_4^{2-} reducers
Dessication	Xerophile	Anhydrobiotic	Lichens, cyanobacteria; arid deserts
Radiation	Radiophile	Ionizing radiation to 15 kGy	*Deinococcus radiodurans*[f]
Pressure	Piezophile[g]	Pressure-loving	Obligate strain MT41;[h] 100 MPa
Chemical extremes	Gases		*Cyanidium caldarium;* pure CO_2
	Metals	Metalotolerant	*Ferroplasma acidarmanus*

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SOURCE: Adapted, by permission from Macmillan Publishers Ltd., from L.J. Rothschild and R.L. Mancinelli, "Life in Extreme Environments," *Nature* 409:1092-1101, 2001, Copyright 2001.

Survival of Organisms in Geological Deposits

Studies of the biology of extreme terrestrial environments have also revealed the potential for long-term survival of viable organisms in ancient geological deposits. At high temperatures, the survival of biological materials is comparatively short, owing to the low thermostability of biomolecules (e.g., ribose and other sugars).[12] Conversely, viable organisms have been retrieved from ancient salt crystals dated at 250 million years old,[13,14] as well as from antarctic and siberian permafrost believed to be millions of years old.[15,16,17] The combined effect of salts and ice is a dramatic reduction of water activity (a_w), which is now considered to permit the maintenance of metabolism at $a_w = 0.3$.[18] This level is considerably lower than previous estimates of $a_w \sim 0.61$ for the water activity limit of biological activity.[19] Regardless, the precise impact of extreme low temperature and water activity stress (or other physicochemical environmental parameters for that matter) on the long-term survivability of viable organisms has still not been established with any degree of certainty.

Resistance to Radiation

Although ionizing radiation is detrimental to the survival of living organisms and is employed as a common method of sterilization, some microorganisms have evolved adaptations to survive under high radiation. Perhaps the most widely known adaptation to radiation is that exhibited by *Deinococcus radiodurans*, which achieves a high degree of resistance by combining multiple copies of its genome with highly efficient DNA repair mechanisms.[20] Less well appreciated is the fact that microorganisms living in a variety of mineralizing environments protect themselves against high doses of ultraviolet radiation with biomediated mineral coatings (e.g., silica, iron oxides, and so on; Figure 3.1).[21] Similarly, endolithic microorganisms—i.e., those residing within the interior spaces of porous rocks and sediments—are afforded protection from radiation, desiccation, and extreme fluctuations of temperature by enclosing mineral matrices.[22]

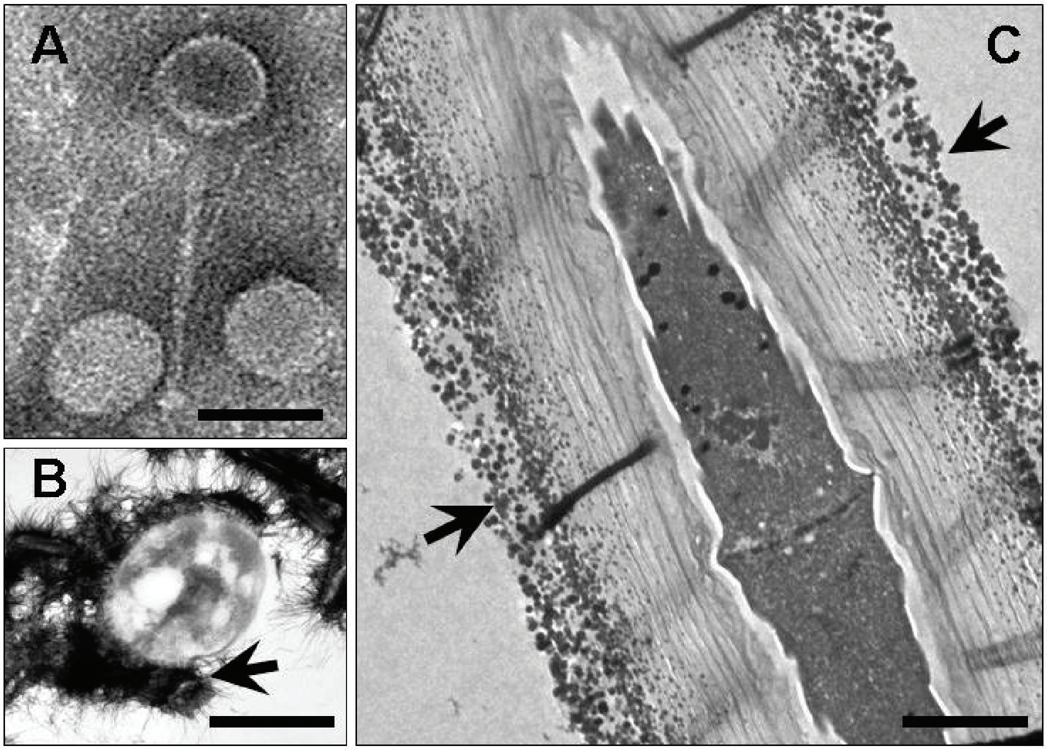

FIGURE 3.1 Transmission electron micrographs of the biomediated mineral coatings that can form around microorganisms in mineralizing environments. A—A microbial virus (bacteriophage) from the acidic (pH 2.3) Rio Tinto, Spain. Scale bar = 0.10 µm. For related information, see J.E. Kyle, K. Pedersen, and F.G. Ferris, "Virus Mineralization at Low pH in the Rio Tinto, Spain," *Geomicrobiology Journal* 25:338-345, 2008. B—Jarosite (ferric hydroxy-sulfate) mineral precipitates (arrow) on the surface of a bacterial cell from the Rio Tinto, Spain. Scale bar = 1.0 µm. For related information, see F.G. Ferris, L. Hallbeck, C.B. Kennedy, and K. Pedersen, "Geochemistry of Acidic Rio Tinto Headwaters and Role of Bacteria in Solid Phase Metal Partitioning," *Chemical Geology* 212:291-300, 2004. C—Silica mineral precipitates (arrows) on the sheath of a cyanobacterial cell from a hot spring in the Atacama Desert, Chile. Scale bar = 5.0 µm. For related information, see V.R. Phoenix, P.C. Bennett, A. Summers Engel, S.W. Tyler, and F.G. Ferris, "Chilean High-altitude Hot Spring Sinters: A Model System for UV Screening Mechanisms by Early Precambrian Cyanobacteria," *Geobiology* 4:15-28, 2006. SOURCE: Images courtesy of F. Grant Ferris, University of Toronto.

CONCLUSIONS

Consideration of advances in microbial ecology over the past decade led the committee to reach the following conclusions:

- Biological studies have continued to expand the known environmental limits for life and have led to the discovery of novel organisms and ecosystems.
- Some living species on Earth have been shown to survive under conditions of extreme radiation, subfreezing temperatures, high salinity, extremely high and low pH, and cycles of hydration to dehydration present on Mars today.
- The discovery in deep subsurface environments on Earth of microbial ecosystems that are able to survive on inorganic sources of energy has greatly enhanced the prospect of chemoautotrophic life in subsurface environments on Mars.
- Studies have confirmed the potential for the long-term viability of terrestrial microorganisms captured in deposits of some extreme terrestrial environments (e.g., ices and evaporates) that have high relevance for Mars exploration.
- Uncertainties in the current assessment of martian habitability and the potential for the inclusion of living entities in samples returned from Mars may be reduced by continued studies of the metabolic diversity and environmental limits of microbial life.

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4

The Potential for Finding Biosignatures in Returned Martian Samples

Over the past decade, growth in the field of geomicrobiology has significantly advanced understanding of the varied roles that microorganisms play in sedimentary processes and has helped define new approaches for the astrobiological exploration of Mars.[1,2] Specifically, studies in a wide variety of modern and ancient environments on Earth have shown that microbial biosignatures (both chemical and morphological) are commonly and preferentially preserved in certain types of sedimentary deposits. Many of these terrestrial examples have relevance for Mars, because similar environments and processes are thought to have operated there earlier in the planet's history. Of special interest for Mars sample return are the deposits of chemical sedimentary environments, like mineralizing springs and seeps, evaporitive lakes, and sites of fine-grained (e.g., phyllosilicate-rich) sedimentation,[3] that have recently been discovered on Mars and are regarded as important potential sites for future landed missions and possibly sample return. On Earth, the above environments typically provide highly favorable nutrient and energy conditions for the growth and reproduction of organisms. In addition, such environments often provide contemporaneously high rates of mineral precipitation, which favors the formation of protective mineral coatings that can enhance the long-term viability of organisms entombed within minerals and increase their potential for preservation as fossil biosignatures.

The question naturally arises in the context of planetary protection, Why care about long-dead fossil biosignatures? If geological samples are returned from Mars, fossils will pose no hazards and therefore can be ignored for purposes of planetary protection. However, there are sound reasons to want to understand the signatures of life found in ancient geological materials. First, it has now been established with reasonable certainty that some types of microorganisms entombed in aqueous minerals and ices can maintain viability for prolonged spans of time (millions to possibly hundreds of millions of years; see examples cited below). Second, the initial characterization of returned martian samples contained within a sample-receiving facility (Chapters 6 and 7), which will be used to guide subsampling for biohazard testing and to inform decisions about sample allocations for further testing, or release from containment, will require understanding of the nature and origin of any organic matter present, whether derived from inorganic sources, from living or dormant life forms, or from fossils. For these reasons, it is prudent to briefly review some of the studies of biosignature capture and preservation that have been carried out in relevant terrestrial environments over the past decade. The number of published studies is substantial, and only a brief review of examples is provided here.

There have been numerous biosignature studies of terrestrial environments that have been considered analogs for Mars, particularly early in the planet's history. Such studies have helped refine strategies for the astrobiological exploration of Mars, while defining new approaches for life detection.

Studies of hydrothermal springs over a broad range of pH and mineralogy (including siliceous,[4,5,6] travertine,[7,8] and iron oxide-precipitating systems,[9,10]) have shown that biosignatures of thermophiles are commonly captured and preserved in both surface and subsurface hydrothermal deposits.[11] In addition, a variety of studies have revealed good organic preservation in deposits of low-temperature surface springs and streams, over a broad range of pH from acidic[12,13,14] to alkaline.[15,16] Studies of modern and ancient evaporite deposits have also shown that microorganisms and their remains are commonly entrapped in a variety of salts, particularly sulfates and halides, both as solid inclusions and within fluid inclusions (Figure 4.1; see also Figure 6.2).[17,18,19,20,21,22] In addition, studies of sedimentary rocks that have experienced significant diagenesis, or alteration under low- to medium-grade metamorphism, also retain fossil organic materials (e.g., kerogen).[23] Microbial biosignatures are not restricted to surface deposits but have also been described from mineralized subsurface fractures and other void spaces in subsurface volcanic rocks.[24,25] Finally, glacial ice and permafrost have been shown to harbor a broad range of extant and dormant life forms, as well as their cryopreserved fossil remains within water and brine-filled voids.[26,27]

The recent discovery of sulfate-rich evaporite deposits by the *Opportunity* rover (see Figure 2.2) and both Mars Express and the Mars Reconnaissance Orbiter have elevated scientific interest in evaporite deposits as potential targets for a martian fossil record (Chapter 2). Similarly, recent orbital detections of phyllosilicates at many locations on Mars[28] have stimulated interest in Mars-analog studies of clay-rich sedimentary systems, which on Earth often provide favorable conditions for the preservation of organic materials.[29,30] Hydrated forms of silica have been shown to precipitate in a variety of aqueous settings, ranging from hydrothermal springs to low-temperature weathering environments. In terrestrial hot springs, silica has been shown to be a particularly favorable medium for preserving organic remains.[31] Recent detections of amorphous hydrated silica (opal) by the *Spirit* rover[32] and

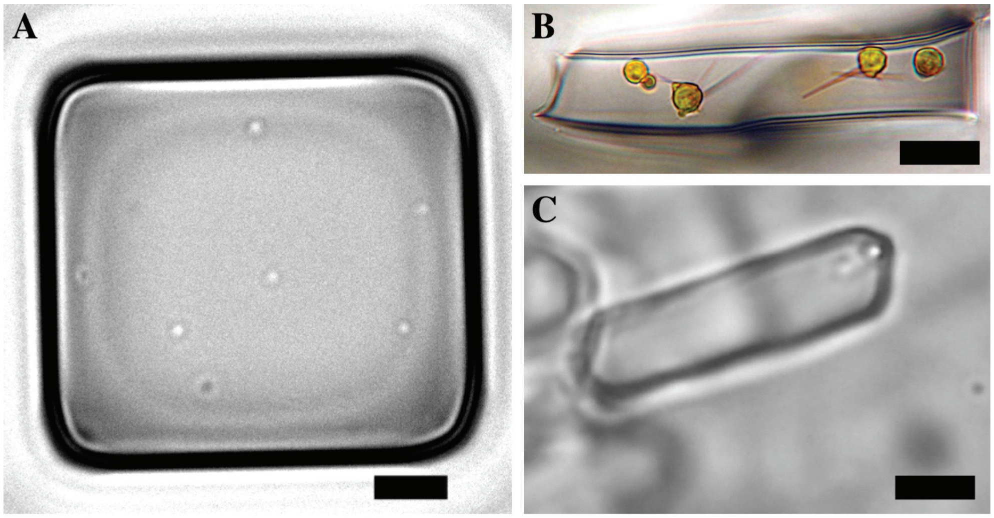

FIGURE 4.1 Microorganisms in fluid inclusions in evaporate crystals. A—Several microbes in fluid inclusions in 31,000-year-old halite from 16.7-meter depth in a core from Death Valley. Scale bar = 2 µm. SOURCE: Image courtesy of Brian Schubert, Binghamton University. B—Yellow algae in a fluid inclusion in modern halite from an acid saline lake in Western Australia. Scale bar = 10 µm. SOURCE: Photograph courtesy of Tim Lowenstein and Michael Timofeeff, Binghamton University. C—Suspect microorganisms (one in focus and one out of focus) in a fluid inclusion in modern gypsum from an acid saline lake in Western Australia. Scale bar = 2 µm. SOURCE: Courtesy of Kathleen Benison, Central Michigan University.

the Mars Reconnaissance Orbiter[33,34] have underscored the need to better understand the range of conditions under which silica deposits form and the potential that silica-rich deposits have to capture and preserve biosignatures.

Hydrothermal springs, seeps, and fumaroles have also been identified as important analogs for Special Regions on Mars.[35] On Earth, hydrothermal springs support highly productive microbial ecosystems that often coexist with high rates of mineral precipitation.[36] This situation favors the entombment of numerous microorganisms and their bioproducts as microfossils, as distinctive biologically mediated microfabrics, and as mesoscale biosedimentary structures, called microbialites.[37] The long-term viability of microorganisms entombed in such deposits is an interesting area for future research. In addition, circulating crustal fluids have the potential to entrain deep-subsurface organisms and deliver them to surface environments where they may be captured and preserved in a viable state within associated mineral deposits and/or ground ice or permafrost (Figure 4.2).[38]

Equally interesting from the standpoint of Special Regions are recently discovered evaporite deposits on Mars,[39,40] including possible halite salts, which are the current record holders on Earth for the prolonged preservation of entombed, viable microorganisms.[41,42] Finally, biosignature studies of martian analog environments and materials on Earth have also stimulated the development of new instrument and payload concepts to support future life detection missions on Mars.[43,44,45]

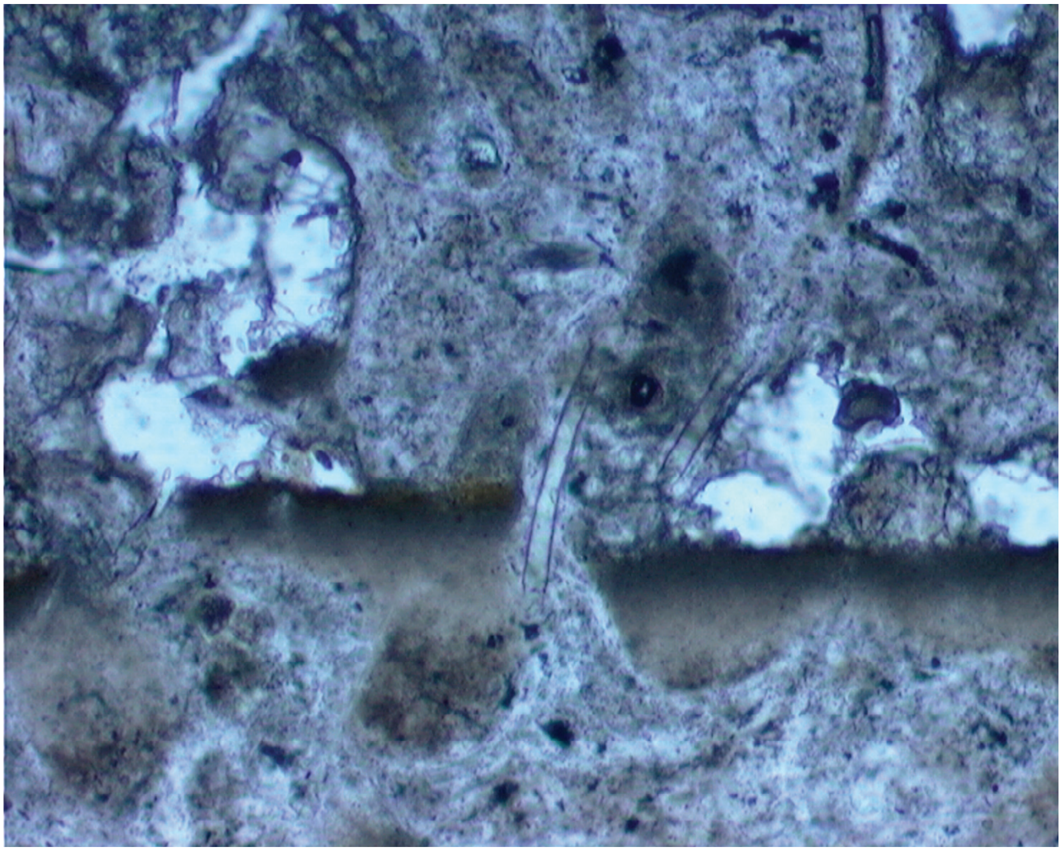

FIGURE 4.2 Photomicrograph of a thin section of a siliceous hot spring (sinter) deposit from the Midway Geyser Basin, Yellowstone National Park, Wyoming, showing heterogeneous microscale fabrics and the fossilized remains of filamentous bacteria. The image is approximately 0.63 mm across. SOURCE: Photograph courtesy of Jack D. Farmer, Arizona State University.

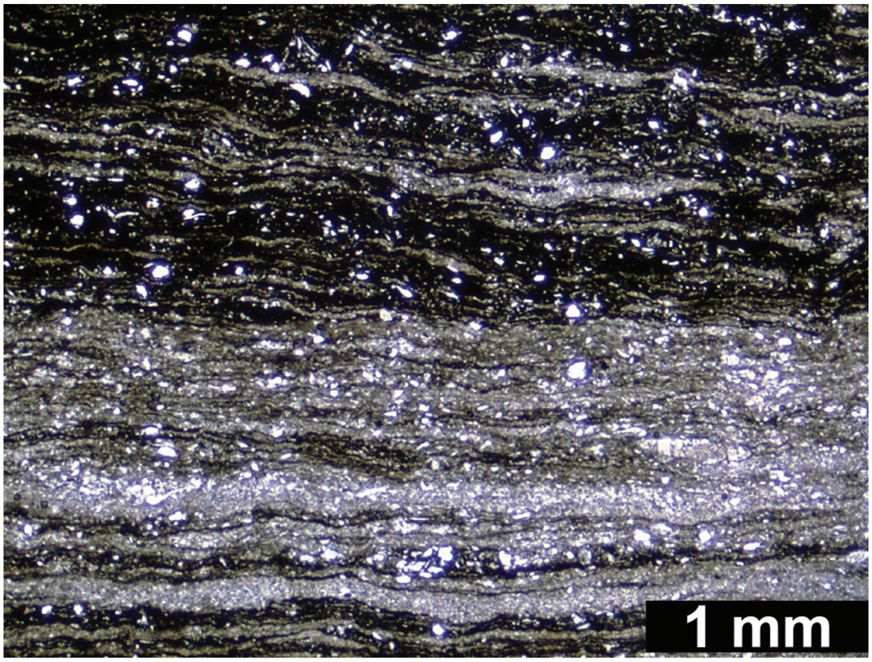

FIGURE 4.3 Photomicrograph showing a petrographic thin-section view of a finely laminated, carbonaceous shale from the Proterozoic Kajrahat Black Shale of the Vindhyan Supergroup, India. Dark laminae are enriched in organic carbon of microbial origin. SOURCE: Image courtesy of Jürgen Schieber, Indiana University; see http://www.shale-mudstone-research-schieber.indiana.edu/. This image was published in J. Schieber, S. Sur, and S. Banerjee, "Benthic Microbial Mats in Black Shale Units from the Vindhyan Supergroup, Middle Proterozoic of India: The Challenges of Recognizing the Genuine Article," pp. 189-197 in *Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record* (J. Schieber, P.K. Bose, P.G. Eriksson, S. Banerjee, S. Sarkar, W. Altermann, and O. Catuneanu, eds.), Copyright Elsevier, 2007.

Many of the same challenges faced in the search for fossil biosignatures in ancient rocks on Earth are directly relevant to the exploration for a fossil record on Mars.[46] Debates over the interpretation of biosignatures preserved in the earliest Precambrian fossil records on Earth[47,48,49,50,51,52,53,54] and putative biosignatures in martian meteorite ALH 84001[55] have stimulated new approaches to fossil biosignature analysis[56,57,58] and directly influenced approaches to Mars exploration.[59] Investigations of Earth's ancient geological record have also provided access to a geological record of ancient environments that were likely similar to early, potentially habitable, martian environments.

It is generally assumed that life may have existed on Earth for more than 3.8 billion years.[60, 61,62] However, proving a biological origin for physical and chemical features found in ancient rocks is often challenged by poor preservation, or confusion arising from morphological and/or chemical convergence between biological and non-biological features and processes (Figure 4.3). This is well illustrated by the persistence of debates over putative signs of life in martian meteorite ALH 84001.[63] The identification of definitive fossil biosignatures in martian samples could prove equally controversial, thus justifying the importance of Mars sample return.

It is important to point out that biosignature research is a field that is still in its infancy. Although the pursuit of multiple lines of evidence consistent with biology may be a sufficient approach to biosignature analysis on a planet where life is both widespread and abundant, this strategy could prove insufficient for resolving questions of biogenicity with materials of extraterrestrial origin, as is well illustrated by the controversy over biosignatures in ALH 84001.

CONCLUSIONS

Geobiological studies of both modern and ancient Mars-relevant environments on Earth have highlighted the potential for samples returned from Mars to contain viable microorganisms or their fossilized remains, while supporting the development of new approaches for in situ and laboratory detections of biosignatures in a variety of geological materials.

The committee found that uncertainties in the current assessment of martian habitability and of the potential for the inclusion of living entities in samples returned from Mars might be reduced by continuing research and development in the following areas:

- Investigations of the prolonged viability of microorganisms in geological materials;
- Studies of the nature and potential for biosignature preservation in a wide range of Mars-analog materials;
- Evaluation of the impacts of post-depositional (diagenetic) processes (deep burial, impact shock, subfreezing temperatures) on the long-term retention of biosignatures in ancient geological materials;
- Definition of reliable criteria for the definitive identification of biosignatures in ancient materials; and
- Development of new laboratory-based and in situ analytical approaches to biosignature analysis.

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5

The Potential for Large-Scale Effects

Interpretations of the discoveries made during the past decade of Mars exploration indicate a significantly enhanced potential for habitable surface environments in the past, as well as the potential for habitable conditions in the deep subsurface today. At the same time, new discoveries of extreme biological systems on Earth have dramatically expanded the known environmental limits for life, opening the range of potentially habitable conditions. While the existence of habitable conditions provides no guarantee that life ever originated on Mars, the possibility has increased that a martian life form, whether active, dormant, or fossil, could be included in a sample returned from Mars.

But these scientific advances have been mirrored by increasing skepticism among the public at large about the risks posed by scientific and technological activities. Controversies concerning, for example, the release of genetically modified organisms into the environment or the intentional or accidental release of exotic pathogens from an increasing number of high-level biocontainment facilities is likely to play some role in any public discussion relating to Mars sample return in general and to a sample-receiving facility (SRF) in particular. Thus, a key question posed to the committee is whether a putative martian organism or organisms, inadvertently released from containment, could produce large-scale negative pathogenic effects in humans or have a destructive impact on Earth's ecological systems or environments.[1]

TYPES OF LARGE-SCALE EFFECTS

The potential effects that are of concern about biohazards can be divided into three broad categories:

- Large-scale negative pathogenic effects in humans;
- Destructive impacts on Earth's ecological systems or environments; and
- Toxic and other effects attributable to microbes, their cellular structures, or extracellular products.

These concerns are addressed in the following sections.

Pathogenic Effects

Understanding of pathogenesis and the nature of biological epidemics has expanded significantly in recent years.[2,3] However, the potential for large-scale pathogenic effects arising from the release of small quantities of pristine martian samples is still regarded as being very low. Significant changes have been made in requirements for containing both known pathogens and novel, or unknown, biological materials, and there have been major improvements in containment design, laboratory practices, and operational oversight.[4,5,6] Numerous reports for planning a Mars sample return mission have acknowledged that biocontainment requirements and planetary protection controls will be integrated as essential elements for handling and testing returned samples.[7,8,9,10]

As reviewed in Chapter 3, extreme environments on Earth have not yet yielded any examples of life forms that are pathogenic in humans. However, it is worth noting in this context that interesting evolutionary connections between alpha proteobacteria and human pathogens have recently been demonstrated for natural hydrothermal environments on Earth,[11] suggesting that evolutionary distances between nonpathogenic and pathogenic organisms may be quite small in some instances. It follows that, since the potential risks of pathogenesis cannot be reduced to zero,[12] a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols.

Ecological Effects

New discoveries in environmental microbiology continue to expand understanding of the taxonomic and metabolic diversity of the microbial world, yet much remains unknown.[13] It is worth noting, however, that extreme environments on Earth have not yet yielded any examples of life forms that are disruptive to ecosystem functions. The risks of environmental disruption resulting from the inadvertent contamination of Earth with putative martian microbes are still considered to be low. But since the risk cannot be demonstrated to be zero, due care and caution must be exercised in handling any martian materials returned to Earth. The demand for a conservative approach to both containment and test protocols remains appropriate.

Toxicity and Other Potential Effects

Although negative effects from nonreplicating biological materials (e.g., toxins and other metabolic by-products) are possible, they are unlikely to be responsible for large-scale pathogenic effects.[14] Nonetheless, they are important as potential biohazards that must be considered when designing protection for the workers who will handle returned martian materials. Operationally, the committee anticipates that existing regulatory frameworks (e.g., that of the Occupational Safety and Health Administration and the Centers for Disease Control and Prevention), coupled with rigorous laboratory biosafety controls, will be incorporated into future discussions of handling and testing protocols and other operations used in the analysis of returned martian materials.

THE QUESTION OF PANSPERMIA

Martian meteorites hold additional importance for planetary protection considerations, beyond the information they convey about environmental conditions on Mars (see Chapter 2). If life originated on Mars and still persists there today, it is possible that over geological time, organisms may have been intermittently delivered to Earth from Mars via impact ejection, a process known as panspermia.[15] Thus, it is appropriate to ask if this natural transfer of materials between Mars and Earth (and vice versa) may have caused large-scale effects for Earth's environments in the past. If large-scale effects have not demonstrably occurred in the past, can the presence of martian meteorites on Earth be used to argue that there are no back-contamination concerns associated with a Mars sample return mission?

The Flux of Martian Meteorites

The rate of influx of martian meteorites to Earth can be estimated only crudely. Roughly 500 meteorites larger than 0.5 kilogram are thought to fall on Earth every year, but only about four are actually observed because most fall into the ocean, or into sparsely populated areas.[16,17,18] Of the 210 meteorites observed to fall between 1815 and 1960, in densely populated areas of Japan, India, Europe, and North America, three were from Mars. Thus, the ratio of martian meteorites to total meteorites is thought to be roughly 1:100. This number is very approximate. So far, about half a dozen martian meteorites have been identified among the 8,000 meteorites recovered from Antarctica. However, considerable analysis is required to identify a martian origin, and most of the antarctic meteorites from Mars have received only cursory examination. If the 1:100 ratio is accepted as being representative, then of the roughly 500 meteorites that fall on Earth every year, perhaps five are from Mars. Because meteorites resemble terrestrial rocks, they are usually recovered only under special circumstances, such as when they have been observed to fall, or by the accumulation of dark-colored meteorites on natural, light-toned surfaces (e.g., accumulation by ablation of the antarctic ice sheet, or aeolian erosion of desert ergs (“sand seas”), like the Sahara, or exposure on playa (dry lake) surfaces of evaporite basins, and so on).

The Survival of Organisms Ejected from Mars

A question of major importance with respect to back contamination of Earth by mechanisms of panspermia is whether putative martian organisms could survive ejection from Mars, transit to Earth, and subsequent passage through Earth’s atmosphere. The Shergottites show evidence for significant shock metamorphism; however, the Nakhlites, Chassigny, and ALH 84001[19] show little evidence of shock damage as a result of ejection from Mars.[20] Passage through Earth’s atmosphere heats only the outer few millimeters of a meteorite, and survival of organics in ALH 84001 and of thermally labile minerals in several other martian meteorites indicates that, indeed, only minor heating occurred during ejection from Mars and subsequent passage through Earth’s atmosphere.

Transit to Earth may present the greatest hazard to the survival of any microbial hitchhikers. Cosmic-ray-exposure ages of the meteorites in current collections indicate transit times of 350,000 to 16 million years.[21] However, theoretical modeling suggests that about 1 percent of the materials ejected from Mars are captured by Earth within 16,000 years and that 0.01 percent reach Earth within 100 years.[22] Thus, survival of organisms in meteorites, where they are largely protected from radiation, appears plausible. If microorganisms could be shown to survive conditions of ejection and subsequent entry and impact, there would be little reason to doubt that natural interplanetary transfer of organisms is possible and has, in all likelihood, already occurred.

Assuming that organisms survive ejection, an important obstacle to long-term viability during transport over interplanetary distances (at low temperatures) is the accumulation of genetic damage from natural background radiation emitted from the radioactive minerals present within the host meteorite. In the absence of active DNA repair, a genome such as that of *Deinococcus radiodurans* would be degraded and become dysfunctional (i.e., non-repairable) within 200 million years,[23] rendering the meteorite sterile with respect to living organisms. A relatively radiation-sensitive bacterium like *Escherichia coli* present within a meteorite could easily survive for 6 million years.[24] Of course, any fossilized remains, or remnant biomaterials, would persist intact, providing a potential record of life. It should be noted that martian materials transported to Earth via a sample return mission will spend a relatively short time (less than a year) in space—all the while protected in containers. (Note that researchers have yet to discover compelling evidence of life in any meteorite, martian or otherwise.) Thus, the potential hazards posed for Earth by viable organisms surviving in samples is significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars.

Martian Meteorites, Large-Scale Effects, and Planetary Protection

Impact-mediated transfers of terrestrial materials from Earth to Mars, although considerably less probable than such transfers from Mars to Earth, should also have occurred numerous times over the history of the two planets. Thus, it is possible that viable terrestrial organisms were delivered to Mars at some time during the early history of the two planets. As noted above, it is also possible that *if* life had an independent origin on Mars, living martian organisms may have been delivered to Earth. Although such exchanges are less common today, they would have been particularly common during the early history of the solar system when impact rates were much higher.

Despite suggestions to the contrary,[25] it is simply not possible, on the basis of current knowledge, to determine whether viable martian life forms have already been delivered to Earth. Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered martian rocks. However, the possibility that such effects occurred in the distant past cannot be discounted. Thus, it is not appropriate to argue that the existence of martian meteorites on Earth negates the need to treat as potentially hazardous any samples returned from Mars via robotic spacecraft. A prudent planetary protection policy must assume that a potential biological hazard exists from Mars sample return and that every precaution should be taken to ensure the complete isolation of any deliberately returned samples, until it can be determined that no hazard exists.

CONCLUSIONS

The committee concurred with the basic conclusion of the NRC's 1997 report *Mars Sample Return: Issues and Recommendations*[26] that the potential risks of large-scale effects arising from the intentional return of martian materials to Earth are primarily those associated with replicating biological entities, rather than toxic effects attributed to microbes, their cellular structures, or extracellular products. Therefore, the focus of attention should be placed on the potential for pathogenic-infectious diseases, or harmful ecological effects on Earth's environments.

The committee found that the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero. Changes in regulations, oversight, and planetary protection controls over the past decade support the need to remain vigilant in applying requirements to protect against potential biohazards, whether as pathogenic or ecological agents. Thus, a conservative approach to both containment and test protocols remains the most appropriate response.

A related issue concerns the natural introduction of martian materials to Earth's environment in the form of martian meteorites. Although exchanges of essentially unaltered crustal materials have occurred routinely throughout the history of Earth and Mars, it is not known whether a putative martian microorganism could survive ejection, transit, and impact delivery to Earth or would be sterilized by shock pressure heating during ejection, or by radiation damage accumulated during transit. Likewise, it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life, based on present evidence.

Assessing the potential for impact-mediated interchanges of viable organisms between Earth and Mars remains an active area of research that may eventually lead to a more refined understanding of the potential hazards associated with Mars sample return. Thus, the committee encourages continued support for research to assess the potential for impact-mediated interchanges of viable organisms between Earth and Mars.

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6

Sample Containment and Biohazard Evaluation

As outlined in previous reports, there is a broad consensus in the scientific community that samples collected on Mars and returned to Earth must be contained and treated as potentially biologically hazardous until they are declared safe by applying recommended protocols, including rigorous physical and chemical characterization, life detection analyses, and biohazard testing. It is important to emphasize that the high level of containment recommended for the handling and testing of martian samples is based on a deliberate decision to take a conservative approach to planetary protection and not because of the anticipated nature of pristine martian materials or organisms. If anything, however, the discoveries over the past decade about Mars and about terrestrial extremophiles have supported an enhanced potential for liquid water habitats and, perhaps, microbial life on Mars, thus making it appropriate to continue this conservative approach.

A factor potentially complicating the policies and protocols relating to sample containment and biohazard evaluation is the de facto internationalization of a Mars sample return mission. All serious planning for Mars sample return is founded on the premise that the scope, complexity, and cost of such a mission are beyond the likely resources of any one space agency. Although no major issues have arisen to date, the international character of Mars sample return raises the possibility that differences in national policies and legal frameworks might complicate issues relating to sample quarantine policies and biohazard certification.

SAMPLE CONTAINMENT

Samples collected on Mars and returned to Earth pose a unique set of containment requirements. They must be contained in ways that will protect Earth from any potential martian hazards and will protect the samples from terrestrial contamination in order to maintain their scientific integrity.[1] The NRC's 1997 report *Mars Sample Return: Issues and Recommendations* divides sample containment into two distinct and separate components.[2] First, there is a Mars sample return spacecraft subsystem—the sample canister—that houses the samples during their journey from Mars to Earth. Second, there is a containment laboratory—a sample-receiving facility (SRF)—to which the still-sealed sample canister is taken following recovery on Earth. Once inside an SRF, the sample canister is opened so that the samples can undergo initial characterization and biohazard testing.

Once martian materials have been placed inside the sample canister it must be sealed to preserve the scientific integrity of its contents and to ensure that its potentially hazardous contents do not contaminate the terrestrial environment. A critical issue relating to the design of the sample canister concerns the means by which those charged with implementing a Mars sample return mission can demonstrate the integrity of the canister's seal. A discussion of the technical means by which this containment is achieved is beyond the scope of this report. What is of concern to the committee is that an overly prescriptive requirement may be counterproductive (see the section "Conclusions and Recommendations" below)

With respect to an SRF, there is a long, well-documented history of successful biocontainment of pathogenic and infectious organisms in biosafety laboratories and under biosafety conditions. However, such facilities typically use negative-pressure gradients to prevent harmful materials from getting *out*.[3] That is, they are designed to leak *in* and as a result are usually "dirty" both chemically and biologically. Similarly, there is a record of successful containment for maintaining the integrity of extraterrestrial and planetary materials. However, these facilities typically use positive-pressure gradients to prevent contaminants from getting *in*.[4] That is, they are designed to leak *out* and as a result are useless for containing hazardous materials. Currently, no single facility exists that combines containment for both biological and planetary materials as required for an SRF for martian materials. Nevertheless, the integration of functions for handling and testing returned martian materials in a single facility seems both appropriate and feasible, as outlined in the existing draft protocol[5] (see next section). Issues relating to an SRF are discussed in Chapter 7.

BIOHAZARDS TESTING

Following publication of the 1997 NRC report *Mars Sample Return: Issues and Recommendations*,[6] two additional reports, the NRC's *The Quarantine and Certification of Martian Samples*[7] and NASA's *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth,*[8] were published in 2002. The latter report provided a set of protocol release criteria indicating when and under what conditions martian samples could be released from containment in an SRF. Conditions for release include:

1. Self-replicating extraterrestrial life forms or indications of life-related molecules are not present, and
2. No harmful effects to terrestrial organisms and environments are evident in biohazard tests.

Sample materials that have not met these criteria must remain in containment, or first be subjected to a sterilization process involving heat, radiation, or a combination of these agents, to ensure that they are safe for further analyses outside containment. Samples that fail to meet these requirements must remain in containment, and all pristine samples released from containment (regardless of the outcome of biohazards testing) must be properly sterilized. Although detailed protocol planning is not within the scope of its charge, the committee agrees with these general recommendations. The committee was, however, verbally encouraged by NASA's planetary protection officer to raise topics that should be considered in greater depth by subsequent planning groups. The committee's digressions in this area focus on general approaches to sample characterization in the context of future protocol testing and decisions about the intentional release of pristine martian samples from containment.

Although the reports mentioned above identified general requirements for sample handling, containment, testing, and certification for release of martian samples, important details are still being discussed, and conflicting recommendations remain that are in need of resolution. To further refine and expand the Mars sample return draft test protocol, NASA and its international partners plan to undertake a follow-up series of workshops, building on all earlier studies and the initial steps taken at the Mars Sample Return Sample Receiving Facility Workshop, held at the European Space Technology Center in the Netherlands in February 2009. While a detailed discussion of Mars sample return protocols is beyond the scope of the present report, in the course of its discussions the committee identified several topics for more detailed consideration by future protocol planning groups.

The Challenge of Biohazards Testing

The testing of potentially biohazardous (especially disease-causing) biological materials has become a somewhat routine procedure in biocontainment laboratories worldwide. Once sufficient information is available for characterizing and understanding the biological materials in question, informed decisions can be made to downgrade or even eliminate containment requirements, if deemed appropriate. However, it is worth pointing out that high-level biocontainment laboratories (e.g., those classified as biosafety level (BSL) 3 and 4) do not routinely test "unknowns." They test materials that have some indication of being involved in causing disease or symptoms. This is a significant distinction. BSL-3 and BSL-4 facilities do not routinely test soils, rocks, water, and other materials for pathogens unless they are implicated in some form or fashion in a disease. In addition, the testing of pathogens becomes increasingly limited to viral agents of a somewhat known molecular basis once it has reached the highest containment level.

The committee was charged with reviewing earlier criteria for sample release and providing suggestions that can guide protocol-planning activities in the years ahead. It is assumed that subsequent protocol planning will define a rigorous battery of tests that will combine physical and chemical characterization, life detection, and biohazards testing. It is also assumed that the criteria for the release of samples from containment, or for bypassing certain tests, will eventually be specified in detail as well. However, at this juncture a key question concerns whether specific, safe, and scientifically justified approaches can be identified that would allow selected martian materials to be released *prior to the completion of rigorous biohazards testing*.

The NRC's 1997 Mars report indicated that pristine samples of martian materials can be released from containment, prior to completion of the entire battery of tests, provided they are first sterilized. However, a detailed comparison of criteria for release in all subsequent reports (see Table 7.1) reveals conflicting statements with regard to both the approach to be used and the specific criteria for sample release. For example, NASA's draft sample-handling protocol allows the release of filtered, contained gases from an SRF, with no further requirement for processing or sterilization. However, the NRC's 2002 report *The Quarantine and Certification of Martian Samples* says nothing about gases, instead focusing entirely on solid samples.[9] It indicates that if solid samples contain no detectable carbon compounds and no evidence of past or present biological activity, smaller subsamples (aliquots) of untreated samples may be released from SRF containment. In contrast, NASA's draft protocol takes a more conservative approach in stating that, regardless of the outcome of physical and chemical tests or life detection studies, all solid samples must undergo complete biohazard testing before release—unless first sterilized. Such discrepancies will need careful resolution in future protocol-planning efforts.

The present committee further supports the NRC's 1997 recommendation that, once samples have been delivered to an SRF, NASA maintain a conservative approach in implementing the protocol and in making decisions about the intentional release of pristine martian samples from containment.

Presumably, in the time leading up to sample return, there will be continuing refinements in methods for sample handling and in the development of new analytical instrumentation for characterizing and testing samples. There will thus be an ongoing need for periodic review of these advances and their potential impacts on NASA's draft protocol and associated criteria for releasing samples from containment. Future protocol planning groups, scientific advisory committees, the Centers for Disease Control and Prevention and other biosafety and biosecurity agencies, and international partners associated with Mars sample return and an SRF will play important roles in these reviews.

The Problem of Sample Heterogeneity

NASA's draft protocol indicates that martian samples that are shown to contain organic molecules (e.g., amino acids, proteins, and so on) must undergo extensive testing before being released from containment. It is presumed that biohazard testing will require the selection of aliquots taken from larger samples. This raises concerns about how best to obtain representative samples that will yield reliable results during testing. This problem is exacerbated by the fact that the biosignatures of rocks, soils, and ices typically show highly heterogeneous distributions within samples at microscopic scales of observation. This heterogeneity arises from spatial variations in mineral (and elemental) compositions; the sizes, shapes, and sorting of grains; and variations in the sizes, shapes, and distributions of void spaces (e.g., intergranular porosity, dissolution voids, vesicles, and interconnected networks of microfractures; see Figures 2.3, 4.2, 6.1, and 6.2, for example). Because the *living* microorganisms contained in rocks and mineral samples typically reside within voids, or in association with particular mineral phases, sampling

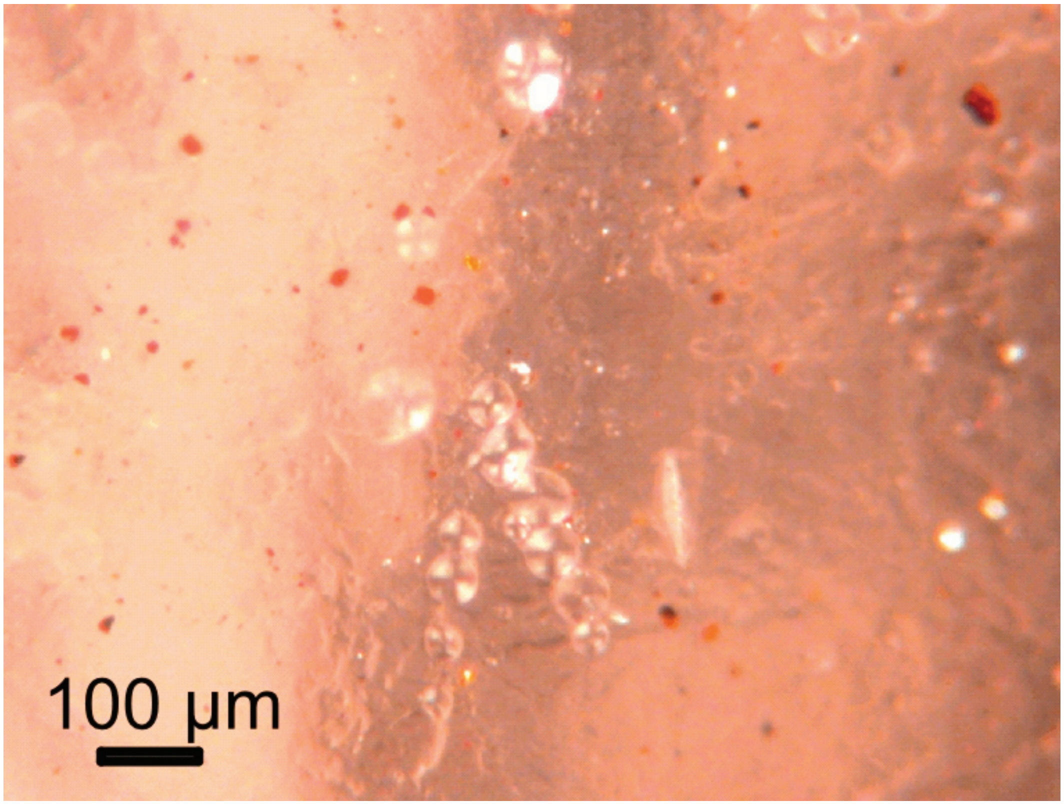


FIGURE 6.1 Thin-section photomicrograph showing mineral heterogeneity. Hematite (orange) and opaline silica (white) are found within host halite crystal from Lake Polaris, Western Australia. Such minerals may provide nutrients for organisms, thereby controlling their microscale distribution in samples. SOURCE: Photograph courtesy of Kathleen Benison, Central Michigan University.

for biohazard testing must carefully consider heterogeneities in the spatial distribution of such features and the impact on the distribution of microorganisms and their by-products within samples.

Especially important as a source of spatial heterogeneity in the microscale distribution of habitable environments that could support living organisms are fluid inclusions—small quantities (~microliters) of aqueous and/or gaseous fluids that are captured during mineral formation. Such inclusions are especially common in aqueous sediments formed by primary chemical precipitation (e.g., evaporites, hot spring sinters, sedimentary cements, fracture fills, ices, and so on—lithologies that have been given a high priority for Mars sample return) or during later aqueous alteration of a mineral. Fluid inclusions found in such deposits frequently contain viable microorganisms or their by-products (see Figures 4.1, 6.1, and 6.2). Thus, it is very important that samples subjected to biohazard testing be acquired from the spatial locations in samples that have the highest potential for containing life or its biosignatures.

The following steps in sample characterization are intended to define a general approach to creating a proper context for subsample selection for biohazards testing:

1. Under primary (SRF) containment, sample exteriors are tested for organic compounds and any released (e.g., possibly biogenic) gases;

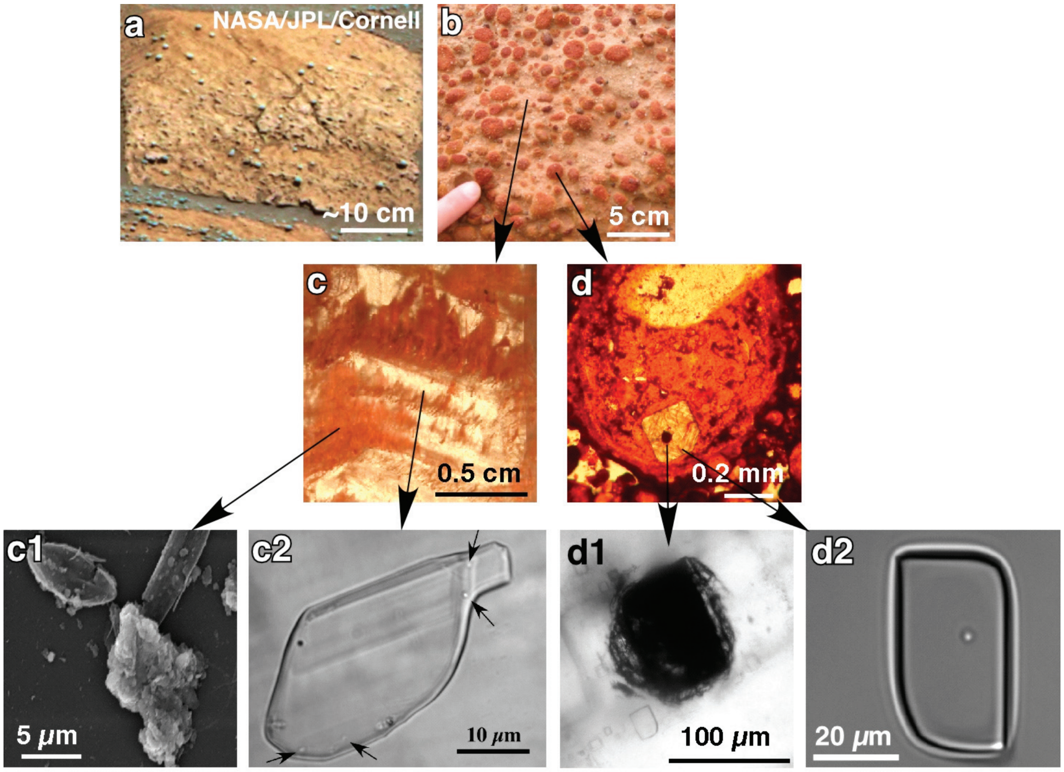


FIGURE 6.2 Examples of heterogeneity in martian and Mars-analog samples from extremely acid saline lakes in Western Australia, as well as living microorganisms and microfossils trapped within such rocks: (a) martian sedimentary rock composed of hematite concretions in a matrix of sand-sized volcanic grains and reworked sulfate grains (see also Figure 2.2); (b) sedimentary rock near acid saline lake in Western Australia composed of red hematite concretions in a matrix of reworked grains of gypsum and quartz coated with hematite; (c) gypsum crystal with included bands of hematite mud, with c1 showing Fe-oxide/Fe-silicate-coated pollen and wood extracted from gypsum and c2 showing microbes within a fluid inclusion in gypsum; (d) interior of hematite concretion, including reworked gypsum (large white grain near top) and halite (white cubic grain), with d1 showing a dark mass consisting of fossil bacteria/archaea and sulfate crystals incorporated in a crystal of halite that formed within the interior of a hematite concretion, and d2 shows a microorganism within a fluid inclusion in a halite crystal. SOURCE: (a) Photograph courtesy of NASA/Jet Propulsion Laboratory and Cornell University; (b, c, c2, d, d1, d2) photographs courtesy of Kathleen Benison, Central Michigan University; (c1) photograph courtesy of Stacy Story, Purdue University.

2. Samples are transported in secondary containers to outside laboratory facilities where nondestructive methods of analysis (e.g., scanning x-ray imaging,[10] tomographic imaging of samples by micro-CT scanning,[11] laser confocal imaging,[12] and synchrotron x-ray photoelectron emission microscopy (X-PEEM)[13]) could be used to map the microscale spatial distributions of minerals and biological elements in samples; and

3. Contained samples are returned to the SRF and microsamples are acquired for biohazards testing from microscopic areas within samples that have been targeted based on compositional and microtextural mapping, obtained in step 2, above.

Given the small sample volumes that are likely to be returned from Mars, it will be important to identify early on the most appropriate approaches for the nondestructive characterization of samples and to support the development of appropriate laboratory facilities. However, implementation of many of the advanced characterization and analytical techniques may not be feasible within the constraints of an SRF. Therefore, a crucial step in defining approaches for sample characterization outside an SRF will be the design of secondary containers for transporting samples to outside laboratory facilities where they can be analyzed (under containment) by a potentially wider range of instruments. Early consideration of the best analytical capabilities will be needed to ensure that flexible, safe designs for secondary containers will be available to ensure proper interfaces with a limited variety of non-SRF laboratory instruments. Optimal designs are likely to require end-to-end testing of a variety of Mars analog materials to refine instrument designs, define necessary instrument sensitivities, and determine minimum sample volumes needed for obtaining reliable results with different types of materials.

CONCLUSIONS AND RECOMMENDATIONS

Planetary protection considerations require that martian materials be securely contained within a sample canister for their journey from Mars, through collection and retrieval on Earth, and subsequent transport to, and confinement in, a sample-receiving facility. With respect to the journey from Mars to an SRF, the NRC's 1997 report *Mars Sample Return: Issues and Recommendations* concluded that the integrity of the seal of the sample canister should be verified and monitored during all phases of a Mars sample return mission. The committee found this requirement to be overly prescriptive. Establishing the technical means to verify containment en route has proven to be a stumbling block in past mission studies. Elaborate steps must be taken to guarantee containment at every stage of the mission. Resources might be better spent in simply improving containment (e.g., by using multiple seals) rather than designing elaborate means of monitoring. The first priority should be to ensure that the samples remain reliably contained until opened in an SRF. The means by which this result is achieved will best be determined by those designing the implementation of a Mars sample return mission.

Recommendation: *The canister(s) containing material returned from Mars should remain sealed during all mission phases (launch, cruise, re-entry, and landing) through transport to a sample-receiving facility where it (they) can be opened under strict containment.*

No facility currently exists that combines all of the characteristics required for an SRF. However, the committee found that there is a long, well-documented history of both the successful biocontainment of pathogenic and infectious organisms and the maintenance of the scientific integrity of extraterrestrial and planetary materials. Thus, the committee concluded that the requirement for handling and testing returned martian materials in a single facility combining biocontainment and integrity-maintaining functions is both appropriate and technically feasible, albeit challenging. In addition, the use of specialty instruments at other facilities may be considered as long as appropriate containment is designed for interfacility transport of pristine materials.

Changes to the requirements for sample containment or criteria for sample release were an issue of concern in the NRC's 1997 Mars report, which recommended that: "The planetary protection measures adopted for the first Mars sample-return mission should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body" (p. 4). The present committee concurs with the spirit of that recommendation with three provisos: first, that the protocols for sample containment, handling, testing, and release be articulated in advance of Mars sample return; second, that the protocols be reviewed regularly to update them to reflect the newest standards; and third, that international partners be involved in the articulation and review of the protocols.

Recommendation: *Detailed protocols for sample containment, handling, and testing, including criteria for release from a sample-receiving facility (SRF), should be clearly articulated in advance of Mars sample return. The protocols should be reviewed periodically as part of the ongoing SRF oversight process that will incorporate new laboratory findings and advances in analytical methods and containment technologies. International partners involved with the implementation of a Mars sample return mission should be a party to all necessary consultations, deliberations, and reviews.*

The NRC's 1997 Mars report recommended that: "Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized" (p. 4). Subsequent NRC and NASA reports have made related, but in some cases conflicting, statements. Irrespective of these conflicts, there are critical issues concerning the selection of the aliquots for biohazard testing and the nature of the tests to be employed.

The discussion of advances in geobiology and biosignature detection in Chapter 4 raises the possibility that viable organisms might be preserved over a prolonged span of time within certain geological deposits. The distribution of extant and fossil organisms and biomolecules in rocks, soils, and ices is heterogeneous at microscopic scales of observation, and this heterogeneity requires careful consideration because it complicates the selection of representative aliquots for biohazards testing.

Recommendation: *Future protocol guidelines should carefully consider the problems of sample heterogeneity in developing strategies for life detection analyses and biohazards testing in order to avoid sampling errors and false negatives.*

The limited amount of material likely to be returned from Mars demands that nondestructive means of analysis be employed to the maximum extent possible in sample characterization and biohazards testing.

Recommendation: *The best nondestructive methods must be identified for mapping the microscale spatial distributions of minerals, microstructures, and biologically important elements within returned martian samples.*

It is highly likely that many of the appropriate nondestructive methods will require the use of techniques whose implementation is not feasible within the confines of an SRF. Thus, a critical issue concerns the design of secondary containers for transporting samples to outside laboratory facilities where they can be analyzed (under containment) using advanced analytic techniques.

Recommendation: *Sample characterization in laboratories outside the primary sample-receiving facility will require the design of secondary containers for safely transporting samples and interfacing with a potentially wider variety of instruments.*

NOTES

1. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002, pp. 52-54.
2. National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997.
3. The atmospheric pressure inside the contained volume is slightly lower than ambient.
4. The atmospheric pressure inside the contained volume is slightly higher than ambient.
5. J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth*, NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.
6. National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997.
7. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002.
8. J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth*, NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.
9. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C. 2002.

10. L. Lemelle, M. Salome, M. Fialin, A. Simionovici, and P. Gillet, "In Situ Identification and X-ray Imaging of Microorganisms Distribution on the Tatahouine Meteorite," *Spectrochimica Acta Part B-Atomic Spectroscopy* 59:1703-1710, 2004.

11. A.A. Kilfeather and J.J.M. van der Meer, "Pore Size, Shape and Connectivity in Tills and Their Relationship to Deformation Processes," *Quaternary Science Reviews* 27:250-266, 2008.

12. K. Kamburoglu, S.F. Barenboim, T. Ariturk, and I. Kaffe, "Quantitative Measurements Obtained by Micro-computed Tomography and Confocal Laser Scanning Microscopy," *Dentomaxillofacial Radiology* 37:385-391, 2008.

13. G. De Stasio, B. Gilbert, B.H. Frazer, K.H. Nealson, P.G. Conrad, V. Livi, M. Labrenz, and J.F. Banfield, "The Multidisciplinarity of Spectromicroscopy: From Geomicrobiology to Archaeology," *Journal of Electron Spectroscopy and Related Phenomena* 114-116:997-1003, 2001.

7

Sample-Receiving Facility and Program Oversight

To achieve the many science objectives that could be accomplished with a Mars sample return mission, it will be necessary to plan, construct, and operate a sample-receiving facility (SRF) with the requisite containment levels, cleanliness conditions, instruments, protocols, and skilled personnel needed to begin the task of fully exploiting the unique opportunities presented by Mars sample return.[1,2]

In the past decade, discussions about Mars sample return missions have advanced considerably, generating a wealth of valuable information that has been applied to problems of sample handling, life detection, and biohazard testing. Initial thinking about Mars sample return, and about an SRF for housing samples, borrowed heavily from lessons learned during the Apollo missions by the Lunar Receiving Laboratory.[3,4,5] Many workshops and studies also have reviewed the science, technologies, methods, and practical issues associated with plans for sample return and testing materials on Earth.[6,7,8,9,10,11]

Additionally, there is a long and successful record of handling extraterrestrial geological samples in ways that maximize analysis and interpretations, without compromising scientific integrity.[12,13] Even recent experiences with the Stardust and Genesis sample return missions have provided useful information and guidance, particularly with respect to the landing, transport, and testing of extraterrestrial materials on Earth.

Other relevant information for sample-return planning has been made possible by the recent rapid expansion of the number of high-level biocontainment laboratories constituting the national biodefense infrastructure. Because they were planned, sited, and constructed as a cohort of containment laboratories, amidst intense public and media scrutiny, they provide valuable lessons of importance for SRF planning and oversight.[14,15,16] These experiences, in combination with various workshops and publications, have provided valuable information concerning the technological, scientific, and other inputs needed for Mars sample return mission planning.

RISK ASSESSMENT

One of the key issues to address in the design of a Mars sample return mission, in general, and an SRF, in particular, relates to the concept of risk. Decisions concerning how to deal with biohazardous materials have to be preceded by a risk assessment. Ultimately, any discussion of the detailed design of a Mars sample return mission and the construction and operation of an SRF will be centered on risk mitigation and reduction. From a biological-hazard perspective, risk is generally a function of hazard (the agent) and the probability that a negative event will occur based on the tasks to be performed with the agent. Consequently, risk-mitigation strategies will focus on eliminating the hazard and/or reducing the probability of a negative event. Both will lead to a risk that is considered acceptable, since achieving zero risk is not possible.

NASA has not yet performed the specific type of risk assessment that might be associated with the design of, for example, a biosafety level (BSL)-4 facility. Such an assessment is premature given that planning for both a Mars sample return mission and an SRF is currently only at the stage of conceptual definition. However, NASA has done a good job so far of considering risk. Issues of biosafety, biosecurity, and biocontainment were discussed at length throughout the process by which the draft protocol was assembled and in reviews and analyses of current methods, instruments, equipment, and facilities used for biocontainment versus planetary science containment. Considerable attention has been paid to the nature of the agent (i.e., pristine martian materials) and to the possible risks associated with it. Finally, U.S. and international experts on biosafety, biocontainment, and risk assessment have participated in many of the past discussions of a Mars sample return mission and an SRF.

TIMESCALE FOR ESTABLISHING A SAMPLE-RECEIVING FACILITY

Although there still is no facility in existence anywhere that combines the requisite biocontainment levels, cleanliness conditions, instrumentation, and other features needed for the characterization and testing of returned martian samples, there is an increasingly clear understanding of what will be required and how it can be accomplished. In addition, existing recommendations provide different viewpoints concerning how and when such an SRF should be established (Table 7.1).

It has been estimated that the planning, design, site selection, environmental reviews, approvals, construction, commissioning, and pre-testing of a proposed SRF will occur 7 to 10 years before actual operations begin.[17,18,19] In addition, 5 to 6 years will likely be required for refinement and maturation of SRF-associated technologies for safely containing and handling samples to avoid contamination and to further develop and refine biohazard-test protocols. Many of the capabilities and technologies will either be entirely new or will be required to meet the unusual challenges of integration into an overall (end-to-end) Mars sample return program.

It will be particularly important to recognize the added lead time needed to establish an SRF while avoiding complications that could jeopardize mission success. Significant planning time for hardware development and testing must be allocated to allow for selection of the best technology concepts among various alternatives proposed and tested. As noted in the iMARS preliminary report,[20] planetary protection and sample receiving are important considerations for designing the Mars sample return mission architecture. These concerns can significantly affect design and time ramifications, with direct and indirect implications for both flight and ground-related mission elements, including control of forward contamination (e.g., to avoid contaminating samples with hitchhiking terrestrial organisms during the collection and packaging of samples on Mars), breaking the chain of contact with Mars, designing a reliable sample container, ground recovery, development of an SRF, sample handling and controls for avoiding contamination, and biohazard-testing protocols. For example, even as a quarantine facility is being planned, there is a need to construct and test mock-ups of clean-room/containment combinations.

The experiences from the Genesis and Stardust sample return missions have demonstrated the increased importance of scrutinizing the entire sample-handling and containment chain, including the landing site characteristics, ground recovery, and transport to ground facilities, not just the quarantine or containment laboratory per se (Stardust and Genesis did not have quarantine laboratories). In addition to technology and hardware developments, it is also important to acknowledge the uncertain lead time that will be needed to accommodate the diverse regulatory review and approval processes that will apply to biocontainment laboratory construction in the post-9/11 era. There is likely to be active public involvement in the decision-making process for a proposed SRF and perhaps even legal challenges that would introduce complications not typically experienced in mission planning.[21] To avoid jeopardizing mission success, there is a strong need to incorporate all aspects of an SRF and sample handling at the earliest stages of Mars sample return mission planning.

TABLE 7.1 Comparison of Major Recommendations Made in Previous Reports from the National Research Council, NASA, and the iMARS Working Group

	Approaches Recommended	
Category	NRC 1997[a]	NRC 2002[b]
Planetary Protection Overall and En Route (Inbound to SRF)		
	No uncontained martian materials may be returned to Earth unless sterilized	Not applicable; (study only considers the handling of materials on Earth)
	If containment not verified en route, must sterilize or not return to Earth	Not discussed
	Containment integrity maintained throughout re-entry and transfer to SRF	Not discussed; report focuses on samples after arrival at SRF
Planetary Protection Measures (Missions)	Planetary protection controls should not be relaxed for future missions without review by an independent scientific body	Not applicable
Assumptions About Martian Life		
Extraterrestrial Life	Martian life might exist and could be returned in samples, but martian organisms unlikely to pose a risk of pathogenic or ecological effects on Earth	Possibility that samples from Mars will contain viable martian microorganisms—which requires that samples be handled in ways that will protect both terrestrial environments and martian samples from any cross-contamination
Biohazards	Samples should be contained and treated as potentially hazardous until proven otherwise; potential biohazards viewed as replicating entities; martian life deemed unlikely to cause infectious, pathogenic, or ecological effects, although the probability is not zero. Subcellular disease agents (e.g., viruses, prions) are biologically part of their host organisms, and extraterrestrial sources of such agents that could affect Earth organisms are extremely unlikely	Agrees with the need to contain and test samples before release; raises concerns that returned samples could include replicating organisms that are self-reliant and able to proliferate in an alien terrestrial world (ignores the potential for viruses, viroids, prions, or other possible biohazards)

NASA 2002[c]	iMARS 2008[d]
Accepts NRC 1997 recommendations and requirements	Accepts requirement of containment and biohazard testing for release
Accepts NRC 1997 recommendations and requirements	Technology developments focus on reliable sample containment throughout all mission phases, including landing, transport of hardware and samples to SRF; operations in SRF until samples are cleared for release; in-flight verification of containment and system-level terminal sterilization
Assumes samples will be returned unsterilized and exterior of sample-return canister will be free of martian materials; container to be opened only in the SRF, followed by rigorous biohazards testing	Assumes draft protocol report will be updated and used to plan SRF; containment to be maintained thoughout re-entry and transfer of samples to SRF
Not applicable	Not applicable
If returned samples include martian life, it may or *may not* be "life as we know it"; the absence of carbon is not evidence for absence of life, but sterilization will be adequate to break the chemical bonds of biological molecules	Not applicable; study focused on facility operations and technology and not issues of science and the potential for martian life
Agrees with the need to contain and test returned samples for biohazards; only replicating organisms or entities that can be replicated and amplified by a terrestrial biological system pose a potential widespread threat. Other potential hazards (e.g., toxins) may be important to consider in protecting laboratory workers exposed to returned samples. Levels of containment and handling in the SRF should be based on perceived risks from biohazards; other potential hazards are dealt with accordingly	Not applicable

Continued

TABLE 7.1 Continued

	Approaches Recommended	
Category	NRC 1997[a]	NRC 2002[b]
Criteria for Release: Controlled Distribution of Unsterilized Materials		
	Samples may be released from containment only if rigorous analyses determine that no biohazards are present, or if subsamples are sterilized first	If samples contain certain or equivocal evidence of martian life, then sterilize to certify for release If samples contain certain or equivocal evidence of life, may transfer to alternate approved facilities, provided all containment and transfer protocols are approved and followed If samples contain no organic carbon compounds and no evidence of past or present biosignatures, can release untreated aliquots from SRF containment without sterilization, for further testing If there is unmistakable evidence of life in samples, they should be dedicated to biological studies (there is also the need to reconsider the optimal study plan and required staffing). In the interim, no releases, unless warranted for biological testing and only if samples sterilized If initial tests are unable to rule out evidence of martian life, or fossilized biosignatures, promptly sterilize aliquots and move samples from SRF to other laboratories for additional biological and geological testing
Decision Making About Release	Decision making for sample release will be based on data from sample characterization, advice of a science advisory committee, and other more specific criteria to be determined	Decision to release samples will be based on the results of protocol and biohazard assessments completed in SRF; science advisory committee to provide guiding recommendations
Sample-Receiving Facility		
Rationale for a Sample-Receiving Facility (SRF)	SRF needed to contain and process returned materials	In agreement that an SRF is needed; SRF must comply with all Centers for Disease Control and Prevention and National Institutes of Health high-containment requirements for BSL-4 laboratories; SRF needs to be able to carry out many functions (unpacking, preliminary examination, baseline characterization, weighing, photography, splitting, repackaging, storage, and sterilization)
SRF Timing	Establish SRF as soon as possible, but at least 2 years prior to launch	Establish 7 years in advance of returned materials; deferring will compromise both quarantine and the scientific study of samples

NASA 2002[c]	iMARS 2008[d]
No solids may be released prior to the preliminary examination of sample materials, with baseline descriptions, cataloguing, and repackaging Subsamples of filtered head gases from the sample container to be made available for distribution beyond SRF without further processing or sterilization Pristine materials only released after physical and chemical, life detection, and biohazards tests are completed and yield no evidence for martian life, or if subsamples are sterilized first Deliberately conservative approach taken (relative to the NRC 2002 report); regardless of the outcome of physical and chemical tests (e.g., carbon content), or life detection tests, all samples should undergo complete biohazards testing before release from containment, unless first sterilized Samples containing any active martian life form, whether hazardous or not, should be kept under appropriate containment, or sterilized before release Samples with life-related molecules require more extensive testing, including biohazards testing, before their release If biohazards tests yield no evidence for living, self-replicating entities, or harmful effects on terrestrial life under Earth conditions, then samples may be released	Not applicable; study recognizes that decisions to release materials reflect both scientific and operational aspects of SRF
Decisions regarding sample release from quarantine to be determined from observational data, and based on advice of a science advisory committee; specific criteria to be refined prior to operation of SRF Gradual reduction of containment level and removal from high containment is possible, depending on the results of biohazards and other tests; contingency plans needed regarding procedures if life is discovered, if test results are equivocal, or if containment is breeched	Not applicable
SRF and protocol objectives: Must contain samples until it is determined whether samples are a threat to Earth's biosphere; SRF needed to implement NRC-recommended sample handling and testing under strict BSL-4 containment and any ambient conditions needed to maintain samples' integrity for scientific analysis; in addition to protocol testing at SRF, must consider environmental, health and safety issues, personnel training, regulatory reviews, and so on.	SRF necessary; details to be determined by others
Commissioning of SRF should occur ~3 years in advance of sample return. Construction and commissioning should be completed at least 2 years in advance of sample delivery to SRF; progressive hiring of personnel and functions should occur before samples are returned	SRF construction and commissioning should be completed 3 years before sample return; ~12 years will be required for the entire SRF planning and implementation process, and ~6 years for maturation of SRF technologies

Continued

TABLE 7.1 Continued

	Approaches Recommended	
Category	NRC 1997[a]	NRC 2002[b]
Sample-Receiving Facility (continued)		
SRF Characteristics and Operations	Not applicable	Design SRF to be small, simple; no science will be done at the SRF that can be done using sterilized materials in outside laboratories Avoid Apollo experiences with vacuum; keep SRF design as simple as possible
SRF Location	Not mentioned; report written under the assumption that NASA will take the lead in an Mars sample return mission	SRF to be located in the United States in affiliation with an existing BSL-4 containment facility, but under NASA control; shared management and operations with international partners; no release before preliminary sample testing is completed
SRF Teams and Staffing	Multidisciplinary science teams will develop and validate procedures for physical and chemical testing, life detection, and biohazards testing, and sample containment and sterilization	Specifics of sample protocol need to be articulated; SRF will require a highly trained cadre of scientists and support personnel; SRF personnel must be able to work in BSL-4 conditions, under containment; pre-training will be required
SRF Advisory (Oversight) Committee	SRF maintained by an advisory panel of scientists; no date stipulated for establishing an SRF (2 years pre-launch?)	SRF will maintain a committee of senior U.S. and international biologists and geochemists to oversee each phase of SRF construction (planning, construction, staffing); advisory committee will also participate in the design of mission elements to address concerns over biological contamination; advisory committee should be established at the earliest stage of Mars sample return planning
Oversight and Related Items		
Intergovernmental Oversight (Planetary Protection Policies and Overall Compliance)	Committee of experts needed to coordinate regulatory responsibilities and advise NASA on planetary protection measures; committee should be in place 1 year prior to the establishment of SRF and 3 years prior to launch	Not specifically mentioned; report focuses on SRF oversight
NASA Administrative Structure	Need to establish a NASA administrative structure to verify and certify adherence to planetary protection requirements at each stage of mission planning	Not specified
Public Communication	Must keep the public openly informed of all plans, activities, scientific results, and any associated issues	Not specified

NASA 2002[c]	iMARS 2008[d]
At a minimum, size and scope of SRF will depend on needs of the sample protocol; ideally, SRF design will be flexible, expandable, and able to adapt functionally; SRF design must consider long-term operations should life be discovered; SRF should support investigator-driven research; some aspects of testing can be done in secondary laboratories, but all must meet containment requirements	Size, scope, and location of SRF to be determined; must provide adequate containment of flight hardware and samples throughout testing for biohazards; should adopt best practices of a BSL-4, plus strict contamination control, especially in the sample-handling chain; will likely need a combination of full-suit lab facility, glove-box lines, robotic manipulation, and a decontamination capability for flight equipment, instruments, and samples
Allows for a variety of SRF strategies and locations; strict containment required; maximize potential for early scientific studies; assumes that primary SRF will be in the United States, with international partners working collaboratively during preliminary testing protocols	Ideally, SRF will be in close proximity to an established, relevant research facility (existing high-containment laboratory, or research cluster); SRF should not be geographically or intellectually isolated
SRF to support investigator-driven research and long-term operations with cooperative agreements with existing BSL-3 and BSL-4 laboratories for personnel training and experience	SRF(s) will maintain multidisciplinary science teams; biosafety officer likely to come from host country
Continuing oversight of SRF planning and implementation by science advisory committee; anticipated that real-time adjustments to the protocol will be required by scientific findings; oversight committee should be established as much as 10 years in advance of Mars sample return; subcommittees needed include a science working group, design committee, and SRF oversight committee	SRF will require an IBC-type oversight committee; oversight needs to be in place several years in advance of the SRF target date for operations; special attention should be given to including international management of the SRF; details to be determined
Review of final protocol should be conducted at the highest scientific levels (e.g., NRC and its international equivalents); oversight should also involve the NASA Planetary Protection Advisory Committee (now the Planetary Protection Subcommittee) and the Mars equivalent of the lunar Interagency Committee on Back Contamination, with multidisciplinary experts from U.S. and international regulatory bodies	Oversight needed; details will depend on the legal framework provided by the host country where SRF is sited; assumes involvement of both U.S. and international partners
Not specified	Not specified
Need to develop a plan for communicating information about Mars sample return, SRF, and scientific findings; plan must be in place well in advance of protocol implementation; advocates a proactive, open dialogue approach; planning should provide guidelines for education and public outreach and details for handling perceived risks and uncertainties	Information about Mars sample return and SRF should be communicated openly to the public; information about SRF will be important for ensuring public confidence

Continued

TABLE 7.1 Continued

	Approaches Recommended	
Category	NRC 1997[a]	NRC 2002[b]
Research Needs/Areas of Research and Development		
	Ongoing in situ surface and orbital studies of Mars needed to identify sites where life could exist, as well as inherently sterile environments; need for more research on extremophiles, martian meteorites, and the potential for dispersal of microbes impact (panspermia); recommends precursor mission for remote sampling of Mars; technology development issues include sample containment and methods for in-flight verification of containment and sterilization and contamination control	Need more research on sterilization and soluble extraction methods for organic compounds in rock matrices prior to sample arrival; recommends immediate testing of mock-ups of containment/clean-room combinations to prove functionality and efficacy

NOTE: BSL, Biosafety Level; NRC, National Research Council; SRF, sample-receiving facility.

[a] National Research Council, *Mars Sample Return: Issues and Recommendations,* National Academy Press, Washington, D.C., 1997.

[b] National Research Council, *The Quarantine and Certification of Martian Samples,* National Academy Press, Washington, D.C., 2002.

[c] J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth,* NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.

OTHER ISSUES ASSOCIATED WITH MARS SAMPLE RETURN

In the years since publication of the NRC's 1997 report *Mars Sample Return: Issues and Recommendations,*[22] there have been numerous proposals for alternative approaches to handling sample return. For example, there has been debate about whether there should be multiple sample-receiving laboratories, rather than a single SRF; the advisability of transporting pristine subsamples to facilities outside the SRF to use special instruments or expertise for testing and sample characterization (see Chapter 6); and whether to site the SRF at a NASA center or in association with an existing BSL-4 containment facility. Discussions have also continued about the requirement to maintain all samples in containment until a full battery of biohazard tests have been completed—and how to accommodate the transport of sample materials to facilities outside the SRF for analysis using specialized instruments. In addition, prospects for international mission partnerships and shared responsibilities for the testing of returned materials have further complicated these discussions.

Clearly, a detailed discussion of these and other issues is beyond the scope of the present report. Suffice it to say that whatever decisions are made about containment and handling, the following planetary protection objectives should be given priority for implementation:

- Maintain the prescribed and appropriate levels of containment for pristine sample materials until a requisite battery of rigorous tests have been completed; and

NASA 2002[c]	iMARS 2008[d]
Need to develop effective sterilization methods and to evaluate effects of sterilization on integrity of geological samples; need to refine methods and measures of sample preparation; raises many specific unresolved issues related to physical and chemical characterization, life detection and biohazard testing; need for regular updates of biohazard testing methods, containment issues, toxicogenomics, refinement of planetary protection and containment guidelines, with exploration of containment options and potential retrofitting of existing containment facilities; need to explore self-contained robotic-handling devices and potential for the miniaturization of analytical instruments; need to develop methods for the re-interrogation of samples at precise locations within samples for diverse testing; need for subsampling procedures and methods for validation/determination of statistical relevance of representative samples selected from heterogeneous materials; need to develop model systems and microcosms for testing of analog and returned sample materials; need to develop robust methods for cell culturing; need life detection methods that can be carried out under simulated martian conditions	Need proper packaging of samples to preserve scientific value of returned samples (e.g., avoiding pulverization, mixing of materials); need to develop methods for avoiding contact transfers of Earth-sourced contaminants (organics, inorganics, and organisms) to sample surfaces; must define end-to-end requirements and analyses for controlling sample cross-contamination; need refinements in the ways that science interfaces with engineering throughout missions; need technological developments that include methods for aseptic sample transfer, redundant containment of flight system hardware, and methods for biohazard testing of samples on Earth

[d] International Mars Architecture for the Return of Samples Working Group, *Preliminary Planning for an International Mars Sample Return Mission: Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group,* NASA, Washington, D.C., and European Space Agency, Paris, France, 2008.

- Preserve, to the maximum extent possible, the scientific integrity of samples during handling and biohazard testing under containment.

In conclusion, as long as containment is maintained at every stage of post-recovery handling and testing and samples are prepared and tracked in appropriate ways, then sample-handling and sample-testing procedures may be accomplished in a variety of ways, perhaps involving multiple laboratories or locations. Regardless of what final decisions are made regarding the containment and handling of returned samples, it will be essential to initiate comprehensive, coordinated planning during the earliest phases of Mars sample return planning.

OVERSIGHT

As already noted,[23,24] the design, construction, and operation of an SRF will require the coordination and work of multiple teams of experts, spanning a decade or more of planning. It will be important for various layers of scientific and technical oversight to be in place early in the planning process to ensure continuity throughout the lengthy and complex Mars sample return mission planning process.

In addition to the establishment of a body to provide scientific and technical advice relating to an SRF, there is also a need for higher-level oversight of all planetary protection requirements associated with Mars sample return. It is clear to the committee that NASA will need to obtain continuing interagency advice (e.g., from the Centers for Disease Control and Prevention and relevant biosecurity agencies and organizations) on planetary protection policies and compliance, similar to the functional role played by the Interagency Committee on Back Contamination (ICBC) during the Apollo program. At present, important advice is provided via the interagency representation on NASA's internal Planetary Protection Subcommittee (PPS). However, the PPS currently reports via the Science Committee of the NASA Advisory Council, an arrangement that could, arguably, lead to conflicts of interest with science and mission efforts. Indeed, the history of the Apollo program, for example, is replete with occasions on which planetary protection concerns and considerations were overruled or ignored when they conflicted with other aspects of mission operations. Appropriate organizational arrangements should be made to avoid such conflicts.

PUBLIC COMMUNICATION AND PROVISION OF INFORMATION

Experience with past and present Mars missions, and with the recent Genesis and Stardust sample return missions, indicates that there will be keen public interest in any program to return martian samples to Earth. In particular, it should be recognized that such a mission is likely to face intense scrutiny regarding the potential risks associated with handling in an SRF on Earth pristine martian materials that could potentially contain extraterrestrial life forms. In addition to concerns about potential biohazards, other issues may arise that are beyond the scope of science and technical realms. Such issues could encompass ethical and legal questions about extraterrestrial life, the implications of either maintaining or sterilizing martian microbes in a laboratory on Earth, and how to communicate findings to the public. All of these concerns necessitate that the public should be openly informed of planning for both a sample return mission and the construction, testing, and operation of an SRF.

CONCLUSIONS AND RECOMMENDATIONS

The NRC's 1997 report *Mars Sample Return: Issues and Recommendations* contained a four-part recommendation relating to various aspects of the establishment and operation of an SRF. The first part concerned the need for such a facility: "A research facility for receiving, containing, and processing returned samples should be established as soon as possible after serious planning for a Mars sample-return mission has begun" (p. 5). Although the present committee supports the intent of this recommendation, it emphasizes that the initiation of planning for an SRF must also include the initiation of planning for, and development of, the activities that will take place there.

Recommendation: *Because of the lengthy time needed for the complex development of a sample-receiving facility (SRF) and its associated biohazard-test protocol, instrumentation, and operations, planning for an SRF should be included in the earliest phases of the Mars sample return mission.*

The second part of the 1997 recommendation discusses the timescale for the establishment of an SRF: "At a minimum the facility should be operational 2 years prior to the launch [of an MSR mission]" (p. 5). The phrase "2 years before launch" is ambiguous in that it could mean 2 years before launch of a Mars sample return mission from Earth or 2 years before the launch of the samples from Mars. More specificity is needed about the duration of the SRF's running-in period and about the activities to be undertaken during that period. In addition, experience with the design, construction, and/or commissioning of new BSL-4 facilities in the United States and overseas (e.g., in the Netherlands, Switzerland, Sweden, and the United Kingdom) suggests that a 2-year running-in period is too optimistic. Facilities may become "operational" at BSL-2 or BSL-3 levels 2 years after completion, but they do not become fully operational as BSL-4 facilities for several additional years. Thus, it is essential to specify that an SRF is fully operational at least 2 years prior to the return of samples to Earth.

Recommendation: *Construction and commissioning of a sample-receiving facility should be completed and fully operational at least 2 years prior to the return of samples to Earth, in order to allow ample time for integrated testing of the facility, the overall test protocol, and instrumentation well in advance of receiving returned martian materials.*

The third part of the 1997 recommendation concerned the roles and responsibilities of the SRF's staff: "The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in the returned samples and for sample sterilization" (p. 5). The present committee concurs with this recommendation.

Recommendation: *A sample-receiving facility should employ multidisciplinary teams of scientists to develop, validate, and perform a rigorous battery of tests that will be used to determine whether and when unsterilized materials returned from Mars may be approved for controlled distribution, or full release from containment.*

The final part of the NRC's 1997 recommendation concerning an SRF dealt with scientific oversight: "An advisory panel of scientists should be constituted with oversight responsibilities for the facility" (p. 5). The committee concurs with this recommendation, but in addition recommends including technical issues relating to an SRF within the oversight committee's terms of reference. The committee's independence should also be specified.

Recommendation: *An independent science and technical advisory committee should be constituted with oversight responsibilities for materials returned by a Mars sample return mission.*

In addition to a science and technical advisory committee for the SRF, the NRC's 1997 Mars report saw a need for a higher-level group charged with oversight of all planetary protection requirements associated with Mars sample return: "A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample-return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least 1 year prior to the establishment of the sample-receiving facility ([i.e.,] at least 3 years prior to launch)" (pp. 5-6). The present committee does not believe that this recommendation is appropriate given the potential conflicts between planetary protection concerns and scientific or operational concerns inherent in NASA's current advisory structure. There is a critical need for the PPS, or its equivalent, and also for the office of the NASA planetary protection officer to be formally situated within NASA in a way that will allow for the verification and certification of adherence to all planetary protection requirements at each stage of a Mars sample return mission, including launch, re-entry and landing, transport to an SRF, sample testing, and sample distribution. Clear lines of accountability and authority at the appropriate levels within NASA should be established for both the PPS (or an equivalent group) and the planetary protection officer, in order to maintain accountability and avoid any conflict of interest with science and mission efforts.

Recommendation: *To ensure independent oversight throughout the lengthy and complex process of planning and implementing a Mars sample return mission, planetary protection policy and regulatory oversight for all aspects of sample return should be provided by both the Planetary Protection Subcommittee (or an equivalent group) and the NASA planetary protection officer, each having suitable authority and accountability at an appropriate administrative level within NASA.*

Finally, the NRC's 1997 Mars report recommended that: "Throughout any sample-return program, the public should be openly informed of plans, activities, results, and associated issues" (p. 6). The present committee concurs with this recommendation and believes that it is also important to explicitly extend the policy of openness to encompass both the sample return mission and the construction, testing, and operation of an SRF.

Recommendation: *The public should be informed about all aspects of Mars sample return, beginning with the earliest stages of mission planning and continuing throughout construction, testing, and operation of a sample-receiving facility.*

NOTES

1. National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997, pp. 30-33.

2. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002, pp. 47-59.

3. J.H. Allton, J.R. Bagby, and P.D. Stabekis, "Lessons Learned During Apollo Lunar Sample Quarantine and Sample Curation," *Advances in Space Research* 22:373-382, 1998.

4. M.S. Race, J.H. Allton, C.C. Allen, and J.Y. Richmond, "Containment Design Concepts for Extraterrestrial Sample Materials," in *Anthology of Biosafety: 1. Perspectives on Laboratory Design* (J.Y. Richmond, ed.), American Biological Safety Association, Mundelein, Ill., 1999.

5. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002.

6. J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth*, NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.

7. D.L. DeVincenzi, J. Bagby, M. Race, and J. Rummel, eds., *Mars Sample Quarantine Protocol Workshop Report*, NASA/CP-199-208722, NASA Ames Research Center, Moffett Field, Calif., 1999.

8. M.H. Carr, ed., *Mars Sample Handling and Requirements Panel (MSHARP): Final Report*, NASA/TM-199-209145, Jet Propulsion Laboratory, Pasadena, Calif., 1999.

9. G.J. MacPherson and the Mars Sample Return Science Steering Group, "Groundbreaking MSR: Science Requirements and Cost Estimates for a First Mars Surface Sample-return Mission," unpublished white paper, 2002, available at http://mepag.jpl.nasa.gov/reports/index.html.

10. G.J. MacPherson and the Mars Sample Return Science Steering Group II, "The First Mars Surface Sample-return Mission: Revised Science Considerations in Light of the 2004 MER Results," unpublished white paper, 2005, available as Appendix III of Science Priorities for Mars Sample Return, posted March 2008 by the Mars Exploration Program Analysis Group at http://mepag.jpl.nasa.gov/reports/ND-SAG_Appendix_IIIpost1.doc.

11. International Mars Architecture for the Return of Samples (iMARS) Working Group, *Preliminary Planning for an International Mars Sample Return Mission: Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group*, National Aeronautics and Space Administration, Washington, D.C., and European Space Agency, Paris, France, 2008.

12. Mars Exploration Program Analysis Group, "Scientific Goals, Objectives, Investigations, and Priorities: 2006" (J. Grant, ed.), white paper, February 2006, available at http://mepag.jpl.nasa.gov/report/index.html.

13. C.R. Neal, "Issues Involved in a Martian Sample Return: Integrity Preservation and the Curation and Analysis Planning Team for Extraterrestrial Materials (CAPTEM) Position," *Journal of Geophysical Research—Planets* 105(E9):22487-22506, 2000.

14. G.K. Gronvall, J. Fitzgerald, A. Chamberlain, T.V. Inglesby, and T. O'Toole, "High-containment Biodefense Research Laboratories: Meeting Report and Center Recommendations," *Biosecurity and Bioterrorism* 5:75-85, 2007.

15. M.S. Race, "Evaluation of the Public Review Process and Risk Communication at High-Level Biocontainment Laboratories," *Applied Biosafety* 13:45-56, 2008.

16. M.S. Race, "Planetary Protection, Biocontainment and Societal Issues: Planning for a Sample Receiving Facility for Returned Martian Materials," presented at COSPAR General Assembly, Montreal 2008, submitted for publication in *Advances in Space Research*.

17. J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth*, NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.

18. International Mars Architecture for the Return of Samples (iMARS) Working Group, *Preliminary Planning for an International Mars Sample Return Mission: Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group*, National Aeronautics and Space Administration, Washington, D.C., and European Space Agency, Paris, France, 2008.

19. National Research Council, *The Quarantine and Certification of Martian Samples*, National Academy Press, Washington, D.C., 2002, pp. 55-56.

20. International Mars Architecture for the Return of Samples (iMARS) Working Group, *Preliminary Planning for an International Mars Sample Return Mission: Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group*, National Aeronautics and Space Administration, Washington, D.C., and European Space Agency, Paris, France, 2008.

21. See, for example, National Research Council, "Technical Input on the National Institutes of Health's Draft Supplementary Risk Assessments and Site Suitability Analyses for the National Emerging Infectious Diseases Laboratory, Boston University: A Letter Report," The National Academies Press, Washington, D.C., 2007.

22. National Research Council, *Mars Sample Return: Issues and Recommendations*, National Academy Press, Washington, D.C., 1997, pp. 30-33.

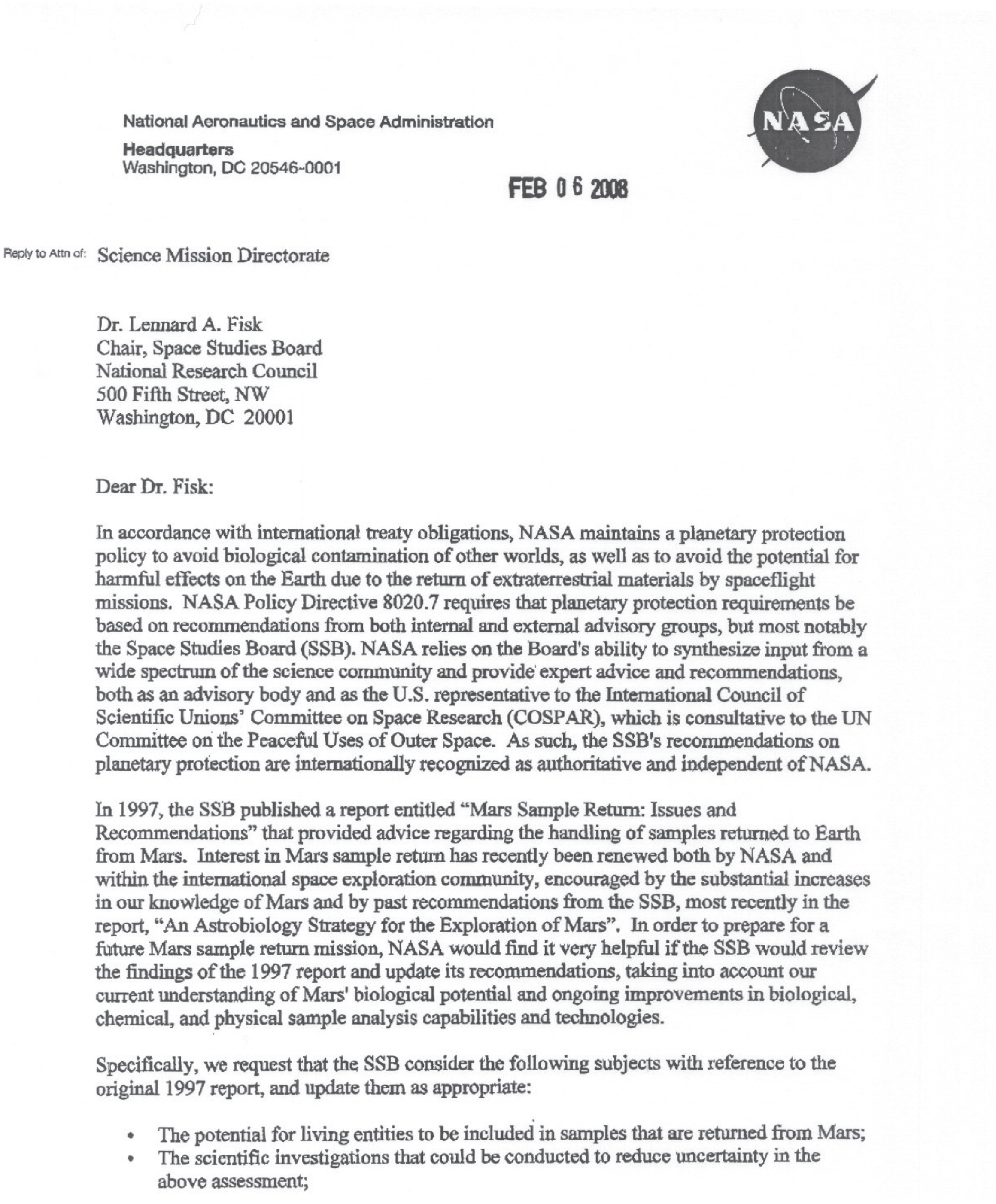
23. J.D. Rummel, M.S. Race, D.L. DeVincenzi, P.J. Schad, P.D. Stabekis, M. Viso, and S.E. Acevedo, eds., *A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth*, NASA/CP-20-02-211842, NASA Ames Research Center, Moffett Field, Calif., 2002.

24. International Mars Architecture for the Return of Samples (iMARS) Working Group, *Preliminary Planning for an International Mars Sample Return Mission: Report of the International Mars Architecture for the Return of Samples (iMARS) Working Group*, National Aeronautics and Space Administration, Washington, D.C., and European Space Agency, Paris, France, 2008.

Appendixes

A

Letter of Request from NASA



National Aeronautics and Space Administration
Headquarters
Washington, DC 20546-0001

FEB 06 2008

Reply to Attn of: Science Mission Directorate

Dr. Lennard A. Fisk
Chair, Space Studies Board
National Research Council
500 Fifth Street, NW
Washington, DC 20001

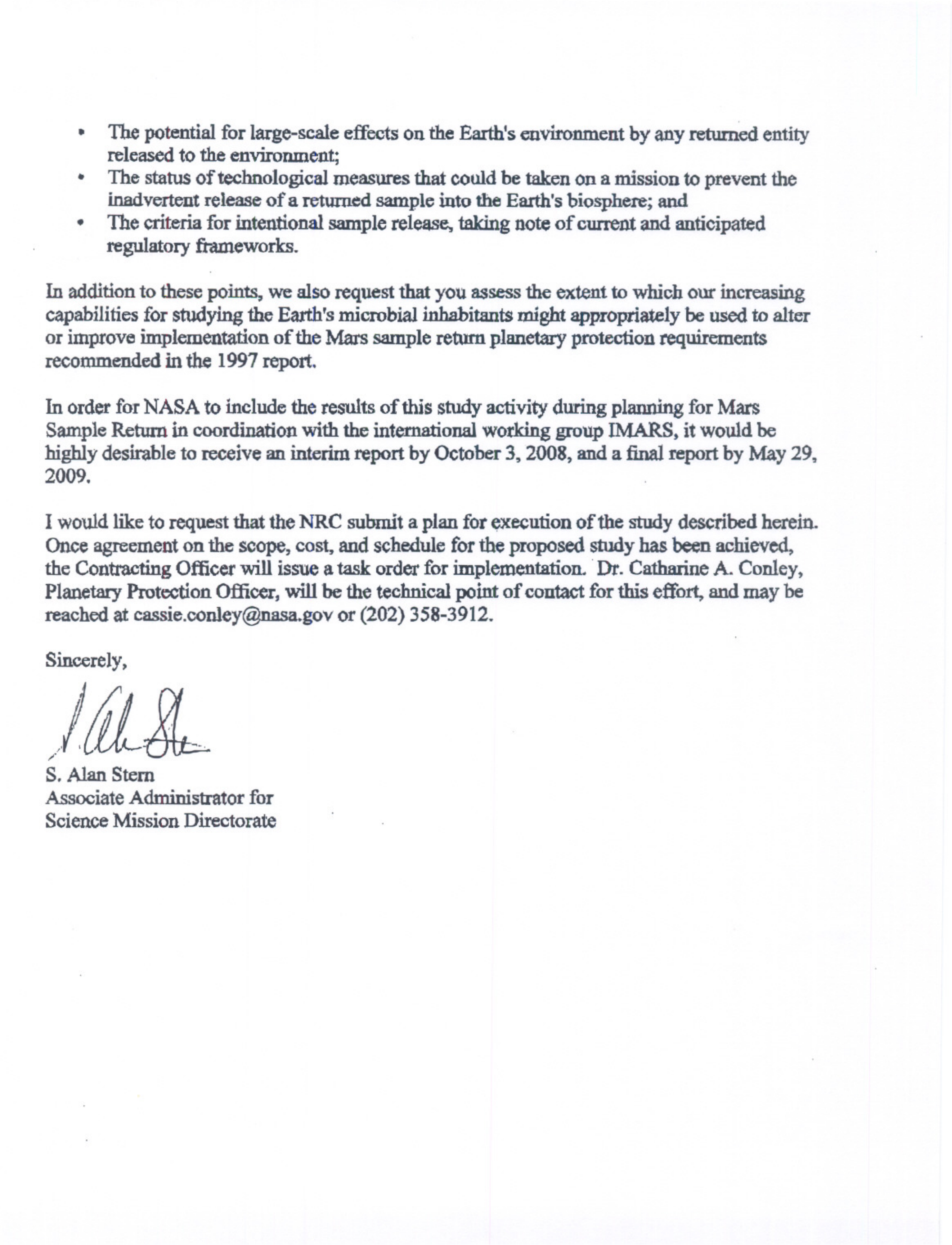
Dear Dr. Fisk:

In accordance with international treaty obligations, NASA maintains a planetary protection policy to avoid biological contamination of other worlds, as well as to avoid the potential for harmful effects on the Earth due to the return of extraterrestrial materials by spaceflight missions. NASA Policy Directive 8020.7 requires that planetary protection requirements be based on recommendations from both internal and external advisory groups, but most notably the Space Studies Board (SSB). NASA relies on the Board's ability to synthesize input from a wide spectrum of the science community and provide expert advice and recommendations, both as an advisory body and as the U.S. representative to the International Council of Scientific Unions' Committee on Space Research (COSPAR), which is consultative to the UN Committee on the Peaceful Uses of Outer Space. As such, the SSB's recommendations on planetary protection are internationally recognized as authoritative and independent of NASA.

In 1997, the SSB published a report entitled "Mars Sample Return: Issues and Recommendations" that provided advice regarding the handling of samples returned to Earth from Mars. Interest in Mars sample return has recently been renewed both by NASA and within the international space exploration community, encouraged by the substantial increases in our knowledge of Mars and by past recommendations from the SSB, most recently in the report, "An Astrobiology Strategy for the Exploration of Mars". In order to prepare for a future Mars sample return mission, NASA would find it very helpful if the SSB would review the findings of the 1997 report and update its recommendations, taking into account our current understanding of Mars' biological potential and ongoing improvements in biological, chemical, and physical sample analysis capabilities and technologies.

Specifically, we request that the SSB consider the following subjects with reference to the original 1997 report, and update them as appropriate:

- The potential for living entities to be included in samples that are returned from Mars;
- The scientific investigations that could be conducted to reduce uncertainty in the above assessment;



- The potential for large-scale effects on the Earth's environment by any returned entity released to the environment;
- The status of technological measures that could be taken on a mission to prevent the inadvertent release of a returned sample into the Earth's biosphere; and
- The criteria for intentional sample release, taking note of current and anticipated regulatory frameworks.

In addition to these points, we also request that you assess the extent to which our increasing capabilities for studying the Earth's microbial inhabitants might appropriately be used to alter or improve implementation of the Mars sample return planetary protection requirements recommended in the 1997 report.

In order for NASA to include the results of this study activity during planning for Mars Sample Return in coordination with the international working group IMARS, it would be highly desirable to receive an interim report by October 3, 2008, and a final report by May 29, 2009.

I would like to request that the NRC submit a plan for execution of the study described herein. Once agreement on the scope, cost, and schedule for the proposed study has been achieved, the Contracting Officer will issue a task order for implementation. Dr. Catharine A. Conley, Planetary Protection Officer, will be the technical point of contact for this effort, and may be reached at cassie.conley@nasa.gov or (202) 358-3912.

Sincerely,

S. Alan Stern
Associate Administrator for
Science Mission Directorate

B

Committee and Staff Biographical Information

JACK D. FARMER, *Chair,* is a professor in the School of Earth and Space Exploration at Arizona State University (ASU). His research interests include microbial bio-sedimentology and the evolution of Earth's early biosphere. He is particularly interested in understanding the factors that control biosignature preservation and how that knowledge can be translated into strategies for the search for evidence of past life on Mars. Prior to joining the faculty at ASU, Dr. Farmer was a research scientist in the Exobiology Branch of NASA's Ames Research Center. He was instrumental in the selection of the landing sites for Mars Pathfinder and the Mars Exploration Rovers. Dr. Farmer served on the science definition team for the Mars Odyssey and Mars Reconnaissance Orbiter missions. He has chaired the NASA Astrobiology Institute's Mars Focus Group and the community-based Mars Exploration Program Analysis Group. Dr. Farmer is a Sequoyah Fellow of the American Indian Science and Engineering Society. He is a past member of NASA's Space Sciences Advisory Committee and has served on several National Research Council (NRC) boards and committees including the Space Studies Board, the Committee to Review the NASA Astrobiology Institute, the Committee for the Review of NASA Science Mission Directorate Science Plan, and the Committee on an Assessment of Balance in NASA's Science Programs.

JAMES F. BELL III is an associate professor in the Astronomy Department at Cornell University. His research interests focus on the geology, geochemistry, and mineralogy of planets, asteroids, and comets using data obtained from telescopes and spacecraft missions. He is particularly interested in the use of optical and infrared techniques to study the surface mineralogy and climatic variations of Mars. Prior to joining the faculty at Cornell in 1995, he was an NRC postdoctoral fellow at NASA's Ames Research Center. Dr. Bell is currently the lead scientist for the Pancam color imaging system on the Mars Exploration Rovers. He is also a member of the science teams for Mars Odyssey, Mars Reconnaissance Orbiter, and the Mars Science Laboratory rover missions.

KATHLEEN C. BENISON is an associate professor in the Department of Geology at Central Michigan University. Her research covers the fields of sedimentary geology and geochemistry. She is also involved with deciphering past conditions on Earth's surface, including depositional environments, paleoclimate, and water chemistry. Her other research covers the physical, chemical, and biological processes of modern sediments that can be compared with ancient sediments.

WILLIAM V. BOYNTON is a professor at the Department of Planetary Sciences at the University of Arizona. Dr. Boynton's research interests include mineralogic and trace element studies of meteorites and impact events, internal stratigraphy and provenance of Cretaceous-Tertiary boundary sediments, remote-sensing via gamma-ray spectrometry, instrumentation for chemical analysis of planetary surfaces, and Mars surface chemistry. He has been extensively involved in Mars missions since 1984. His gamma ray spectrometer first flew on the ill-fated Mars Observer spacecraft in the early 1990s before being successfully deployed by Mars Odyssey in 2002. He is the principal investigator of the Thermal and Evolved Gas Analysis instrument, which studied the chemical properties of martian surface materials on the Mars Phoenix spacecraft. Dr. Boynton served on the NRC Committee on Planetary and Lunar Exploration and the Committee on the Assessment of Solar System Exploration.

SHERRY L. CADY is an associate professor at the Center for Life in Extreme Environments in the Department of Geology at Portland State University. She studies microbial behavior and biosignature preservation in extreme ecosystems to better detect life in the geological record. Specifically, she uses a variety of imaging, structural, and chemical analytical methods and focuses on the biochemical interactions between microorganisms and their environment. Her efforts to improve the ability to detect evidence of life in the geological record apply directly to paleobiological studies of life on Earth and astrobiological studies on other planets. Dr. Cady is the editor of the journal *Astrobiology.* She served as an NRC research associate at NASA Ames Research Center (1994-1996), and as principal investigator and research scientist at the SETI Institute (1996-1998). Dr. Cady served on the NRC Committee to Review of the Next Decadal Mars Architecture.

F. GRANT FERRIS is a professor in the Department of Geology at the University of Toronto and the founding director of the university's Microbial Geochemistry Laboratory. Dr. Ferris' research focuses on field studies of mineral precipitation by bacteria in terrestrial hot springs and deep-sea hydrothermal vents and experimental laboratory work on the surface chemistry of bacterial cells. He serves as the chair of the executive board of the International Symposia on Environmental Biogeochemistry and is a founding member of the Canadian Space Agency Astrobiology Working Group and a member of the InterRidge Biogeochemistry Working Group. He has served as an associate editor for the *Geomicrobiology Journal, Applied Geochemistry,* and *Geobiology*.

DUNCAN MacPHERSON, a Jet Propulsion Laboratory (JPL) fellow, serves in a variety of roles, including chief engineer for spacecraft projects—most recently, the Jupiter Icy Moons Orbiter (2003-2005). Prior to coming to JPL in 2000, Mr. MacPherson worked as an independent contractor providing senior-level consulting services addressing technical problems relating to systems engineering or multiple engineering disciplines. In this capacity Mr. MacPherson served on project-level review boards for numerous spacecraft, including Galileo and Cassini. From 1965 to 1989, Mr. MacPherson held a variety of senior technical positions at Hughes Aircraft Company, including chief engineer for the Galileo Probe Project and the Japanese Geostationary Meteorological Satellite. He was also responsible for systems engineering and mission analyses for classified and unclassified programs and proposals.

MARGARET S. RACE is a scientist with the SETI Institute. Her research focuses on planetary protection and ethical considerations of probes seeking to detect life as well as the implications of the possible discovery of life beyond Earth. She works closely with NASA in studying scientific, policy, and public issues associated with solar system exploration. She has served on three major national studies involving planetary protection and recently completed work on several NASA projects related to Mars exploration—one that developed scientific protocols for handling, quarantining, and testing martian samples, and one that analyzed the technical and scientific issues associated with human missions to Mars. She served as an organizer and editor of a series of international workshops on containment and testing protocols for Mars sample return missions and participated in several recent studies of planetary protection for human missions to Mars. Dr. Race has served on several NRC committees, including the Committee on Principles of Environmental and Scientific Stewardship for the Exploration Study of Subglacial Lake Environments, the Committee on Preventing the Forward Contamination of Mars, and the Task Group on Issues in Sample Return.

MARK H. THIEMENS is a professor of chemistry and biochemistry and dean of the Division of Physical Sciences at the University of California, San Diego (UCSD). He also directs UCSD's Center for Environmental Research and Training. Dr. Thiemens is best known for his discovery of the mass-independent isotope effect, which led to an improved understanding of Earth's atmospheric composition and evolution. In 1998, he received the Ernest O. Lawrence Medal for this discovery. He has developed new insights into atmosphere-surface interaction on Earth and Mars and has stimulated a new approach to theories of isotopic reaction mechanisms. Work in Dr. Thiemens' laboratory has concentrated on measurements of anomalous isotope variations in martian meteorites and in the oldest-known rocks on Earth. Dr. Thiemens is a member of the National Academy of Sciences and serves on the editorial board of the *Proceedings of the National Academy of Sciences*.

MEENAKSHI WADHWA is the director of the Arizona State Center for Meteorite Studies. Her research interests focus on deciphering the origin and evolution of the solar system and planetary bodies through the use of geochemical and isotopic techniques. She uses high-precision mass spectrometric techniques to investigate a wide range of solar system materials. These include meteorites of martian and asteroidal origin, Moon rocks (from the Apollo missions and lunar meteorites), and other samples returned by spacecraft missions such as Genesis and Stardust. Dr. Wadhwa served on the NRC Committee on the Origins and Evolution of Life and the Committee on an Astrobiology Strategy for the Exploration of Mars.

Staff

DAVID H. SMITH joined the staff of the Space Studies Board in 1991. He is the senior staff officer and study director for a variety of NRC activities, including the Committee on the Origins and Evolution of Life and the ongoing planetary sciences decadal survey. He also organizes the SSB's summer intern program and supervises most, if not all, of the interns. He received a B.Sc. in mathematical physics from the University of Liverpool in 1976 and a D.Phil. in theoretical astrophysics from Sussex University in 1981. Following a postdoctoral fellowship at Queen Mary College, University of London (1980-1982) he held the position of associate editor and, later, technical editor of *Sky and Telescope*. Immediately prior to joining the staff of the Space Studies Board, Dr. Smith was a Knight Science Journalism Fellow at the Massachusetts Institute of Technology (1990-1991).

CATHERINE A. GRUBER is an 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. 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.

RODNEY N. HOWARD joined the Space Studies Board as a senior project assistant in 2002. Before he joined SSB, most of his vocational life was spent in the health profession—as a pharmacy technologist at Doctor's Hospital in Lanham, Maryland, and as an interim center administrator at the Concentra Medical Center in Jessup, Maryland. During that time, he participated in a number of Quality Circle Initiatives that were designed to improve relations between management and staff. Mr. Howard obtained his B.A. in communications from the University of Maryland, Baltimore County, in 1983.

KAYLEIGH BOHEMIER, Lloyd V. Berkner Space Policy Intern, attends Smith College, where she majors in English and minors in astronomy. While at school, she works with the Astronomy Department as a teaching assistant for introductory laboratory classes. This semester, she is studying abroad at Royal Holloway University of London. Ms. Bohemier discovered her interest in space policy when she read *Venus in Transit*, a work that exposed her to the extensive political drive behind astronomical research and development, shortly after a grassroots work experience. She plans on pursuing a master's degree in science and technology studies or science policy.