BIOLOGICAL
CONTAMINATION OF
MARS
ISSUES AND
RECOMMENDATIONS

Task Group on Planetary Protection

Space Studies Board
Commission on Physical Sciences, Mathematics, and Applications
National Research Council

Cover: This Viking Orbiter image, 200 kilometers across, shows water-worn, branching valley networks in the cratered uplands of Mars. These valleys are the main evidence for a warm wet climate on early Mars. (Photograph courtesy of NASA.)

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INTRODUCTION

Task, Approach, and Scope of Report

Whenever Earth-originating spacecraft intrude on the atmosphere or surface of other solar system bodies or return to Earth from one of these bodies, there is a risk of contamination by foreign substances or organisms. In the case of in situ exploration of other bodies, a major concern is disruption of scientific findings by imported material. In the case of back contamination (return to Earth of extraterrestrial material), there is concern over the possible release into the biosphere of potentially harmful organisms or substances.

Since 1967, a policy of planetary protection has been in place in order to control contamination of planets by terrestrial microorganisms and organic constituents during planetary missions. In the United States, the policy is implemented by the National Aeronautics and Space Administration (NASA). It is accepted as official policy by the Committee on Space Research (COSPAR) of the International Council of Scientific Unions. The policy lays out a framework of specific planetary protection guidelines for implementing procedures for future missions. Through COSPAR, review and analysis of the policy have been ongoing and have resulted in periodic revisions in light of new information obtained from planetary exploration.[1,2]

In addition, the United States is a signatory to an international treaty that declares in part that "States Parties to the treaty shall pursue studies of outer space . . . so as to avoid their harmful contamination and also adverse changes in the environment of the Earth . . . ."[3]

The Space Studies Board (SSB) of the National Research Council has served as NASA's primary advisor concerning planetary protection (or quarantine) for many years. The board, through its Committee on Planetary Biology and Chemical Evolution, has published a number of reports and letters concerning planetary protection (or quarantine) in response to NASA requests.[4-12] Most recently, NASA's planetary protection officer requested that, prior to the 1992 COSPAR meeting, the board make recommendations regarding planetary protection policy for upcoming Mars missions (Appendix A). In response to this request, the board formed the ad hoc Task Group on Planetary Protection, made up of planetary scientists, biochemists, ecologists, and microbiologists who specialize in studying life in extreme environments such as the polar regions and deep oceans and lakes (Appendix B). The task group hosted a workshop in September 1991 at which extensive briefings on planned and contemplated Mars missions and the many aspects of Mars science and survival of Earth organisms were reviewed and discussed in detail (Appendix C). Scientists from Europe and the former USSR made presentations concerning their current views and approaches to planetary protection. These presentations and discussions, along with a reassessment of the SSB's 1978 report, _Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan_[13] (excerpted in Appendix D), form the basis for this report. Additional information considered by the task group is given in Appendix E.

In keeping with NASA's request, the task group focused on making recommendations concerning the protection of Mars from forward contamination (i.e., contamination of the martian environment by terrestrial organisms) during upcoming missions by both the United States and the former Soviet Union. In so doing, it distinguished between missions whose goals include reconnaissance and measurement and those that specifically include experiments to detect life. The task group also discussed what additional knowledge will be needed in order to assure that future recommendations regarding contamination of Earth from Mars (back contamination) might be made with a higher degree of certainty than is now possible.

Following a short introduction to the rationale underlying planetary exploration (Chapter 1) is a brief summary of approved and contemplated missions to Mars (Chapter 2). Chapter 3 briefly reviews the state of knowledge in several areas pertinent to the problem of planetary protection, including chemical and physical properties of Mars, and Chapter 4 discusses the limits of life on Earth and the abilities of known terrestrial organisms to withstand extreme environmental conditions, as well as new approaches to detecting life forms. Chapter 5 includes a review and comments—made in light of current knowledge—on the recommendations made in _Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Nep-_

tune, and Titan. Updates to the recommendations made in 1978 are also given in Chapter 5. Chapter 6 gives additional recommendations concerning collection of essential data, spacecraft sterilization and bioburden assessment, and future research, as well as legal and societal issues and NASA's overall planetary protection program.

Background

Understanding the origin and evolution of life has been an important goal of NASA; studies in this area generate some of the more interesting scientific questions for all mankind. One promising approach to understanding life's origins is that of searching for life elsewhere, primarily on other planets, where physical, hydrological, and geochemical properties might favor (or might have favored in the past) the existence of replicating biotic systems like those found on Earth. Historically, Mars has been the planet of choice for understanding life's origins.

With the technological advances that accompanied the advent of spacecraft exploration, our ability to conduct detailed studies of planets in the solar system improved dramatically. As our knowledge of present conditions on the surface of Mars has increased, there has been a concomitant decrease in any expectation that life as we know it could exist on the surface of the planet. At the same time, it is important to remember that (1) Viking lander sites have not been representative of the entire planet and (2) the early state of Mars seems to have differed quite markedly from its present state and may have been characterized by the presence of abundant liquid water and a more substantial atmosphere. Future life-detection missions to Mars must include investigation of other more biologically relevant, desirable sites where evidence of the survival of either molecular or morphologically preserved cells or cell components may exist.

As in the past, it is necessary to continue to take precautions to ensure planetary protection, both from forward and back contamination. With respect to forward contamination, NASA's historic concern has been to preserve pristine conditions on the planets for future experiments with biological and organic constituents that might lead to insights concerning the origin and evolution of life in the cosmos. Knowledge has increased substantially since the Viking mission. Recommendations for planetary protection that guided the Viking mission may not be relevant to missions being flown today or to those planned for the future. As more information is acquired about a given extraterrestrial body, assessment of the amount of planetary protection needed to protect that body from contamination should change accordingly. The process must be iterative and must allow for altering the techniques used to ensure protection as we learn more about planetary conditions and the probability of contamination.

FUTURE MISSIONS

At this time, there are two approved missions to Mars: the U.S. Mars Observer mission to be launched in October 1992 and the Soviet Mars 94/96 mission. Both NASA and the European Space Agency (ESA) are studying a network mission that involves placing numerous small stations on the surface of the planet. In addition, both the United States and the former Soviet Union have been studying various rover and sample return missions for some time. These missions, which will gradually improve our knowledge of the environmental parameters of Mars and enhance our ability to select and protect appropriate landing sites, are discussed in detail in Chapter 2.

SURFACE ENVIRONMENT OF MARS

Despite an incomplete understanding of the surface environment of Mars, it is generally agreed that conditions are extremely inhospitable to terrestrial life. Various aspects of the surface environment have relevance to the issue of forward contamination, including both growth on Mars of organisms from Earth and the lifetime of bioorganic matter deposited on the martian surface. Chapter 3 of this report reviews the state of knowledge regarding the martian surface, including its chemistry, solar radiation flux, temperature, water, volcanism, and past climate conditions.

LIMITS OF LIFE ON EARTH: EXPANSION OF THE MICROBIAL WORLD AND DETECTION OF LIFE

Life in Extreme Environments

The Task Group on Planetary Protection assessed past reports and current views on the range of environmental conditions believed to exist on Mars and unanimously agreed that it is *extremely unlikely that a terrestrial organism could grow on the surface of Mars*. It is clear that the most extreme environments on Earth where organisms can replicate are considerably less extreme than the environments that are known to occur over most of the martian surface. Particularly important in this regard are the high levels of ultraviolent radiation, the thin atmosphere, the extremely low maximum temperatures, and the absence of liquid water on the surface.

Based on current knowledge of conditions on Earth that limit cell growth and on the best estimates of surface conditions on Mars, the task group concluded that no known terrestrial organisms could grow on the martian surface. However, this does not imply that life does not exist anywhere on the planet. There is far too little information to assess the possibility that life may exist in subsurface environments associated with hydrothermal

activity or in selected microenvironments free from the harsh conditions previously mentioned, or to conclude that organisms resembling terrestrial life forms did not evolve on Mars.

The task group concentrated on the problem of forward contamination by intact cells or components of cells that could be detected by sophisticated molecular methods in future expeditions designed to look for evidence of extant or past life on Mars. Planning for present and future missions to Mars must include awareness of new results obtained from studies of extreme environments as well as the inevitable extension of the limits of environments where growth and survival can take place. Advances in understanding the microbiology of extreme environments have been accompanied by advances in the development of new methods and considerably more accurate and sensitive instruments for detecting the presence of life and life-related molecules and for identifying their evolutionary relatedness.

Nevertheless, it is not a straightforward matter to define the ranges of physical and chemical conditions on Earth in which organisms can grow, replicate, or survive for extended periods. During the 13 years since the SSB's last report on planetary protection, *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan*, bacteria have been detected or isolated from many of Earth's hostile environments—the dry, extremely cold subsurfaces and interiors of rocks in the dry valleys of the Antarctic, hot environments associated with submarine and terrestrial volcanoes and geothermal systems, and deep subsurface sediments and aquifers. Chapter 4 includes a review of these organisms.

Life Detection and Bioburden Determination for Planetary Protection

Techniques for assessing the existence of microorganisms have advanced dramatically since pre-Viking days. These advances will have a strong impact both on bioburden assessment procedures and on future life-detection experiments. New methods have been developed with increasingly greater sensitivity and specificity. **The task group strongly recommends that efforts be made to explore current analytical methods for use in bioburden assessment and inventory procedures before spacecraft assembly and launch.**

In addition to epifluorescent microscopic techniques for directly counting viable cells, many other new methods have been developed, such as the polymerase chain reaction, allowing greatly increased sensitivity of detection by enzymatically amplifying specific biomarkers of even a single cell to detectable levels. The appeal of these techniques is their extreme sensitivity. In many cases, single cells can be detected and identified with confidence.

ASSESSMENT OF THE 1978 REPORT

Review

Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan, the 1978 report by the then Space Science Board's Committee on Planetary Biology and Chemical Evolution, established a quarantine policy for exploratory, one-way missions to Mars, Jupiter, Saturn, Uranus, Neptune, and Titan planned for 1974 to 1994. The task group's assessment of this report is limited to an evaluation of information and past recommendations concerning Mars. After the 1978 report was issued, NASA began to look for ways to simplify planetary protection procedures as they applied to particular upcoming planetary missions, and to minimize the use of mathematical models.

Prior to the 1978 report, the criteria used for determining categories of planetary contamination were those established by international agreement through COSPAR. They stipulated that the probability of contamination (P_c) should be less than 1×10^{-3} for each planet. Considerable uncertainty was engendered by this probabilistic approach to planetary protection. Concern related to this point has been expressed over the years by virtually every group that has analyzed the problem, and indeed by NASA. Although the probability of depositing a microbe or some organic material indicative of life is very high (microbes and organic contaminants have almost certainly been deposited by past missions), our expectations regarding the likelihood of permanent contamination as a result of microbial growth (expressed as the probability of growth, P_g) have been steadily reduced as we have learned more about Mars.

The NASA studies that followed the 1978 report culminated in a 1984 report to COSPAR that greatly deemphasized the probabilistic approach and introduced the concept of target planet and mission-type categories.[14] This approach, which is reviewed in Chapter 5, directly reflects the degree of concern for a given planet, in the context of a particular type of mission.

Recommendations of the Task Group

The task group views the problem of forward contamination as separable into two principal issues: (1) the potential for growth of terrestrial organisms on Mars and (2) the importation of terrestrial organic contaminants, living or dead, in amounts sufficient to compromise the search for evidence of past or present life on Mars itself.

The guidelines concerning probabilities of growth (P_g) issued by the Space Science Board in its 1978 report were recently reassessed in a 1991 NASA report.[15] Comments and estimates made by the participants illus-

trate a consensus that the P_g values for terrestrial organisms on Mars are probably lower than the 1978 estimates. However, this observation does *not* alter the case as far as contamination of a possible past or extant martian biosphere is concerned. Prudence dictates that bioload reduction on all lander missions to Mars must continue to be seriously addressed. The issue of spacecraft cleanliness is particularly crucial when life-detection experiments are included in the scientific payload.

The deliberations of the task group were greatly aided by the MESUR mission workshop that resulted in the above-mentioned 1991 report. That report, together with the comprehensive briefings given by experts on relevant matters, led the task group to concur unanimously with the following conclusion from the MESUR workshop:

> Forward contamination, solely defined as contamination of the martian environment by growth of terrestrial organisms that have potential for growth on Mars, is not a significant hazard. However, forward contamination more broadly defined to include contamination by terrestrial organic matter associated with intact cells or cell components is a significant threat to interpretation of results of in situ experiments specifically designed to search for evidence of extant or fossil martian microorganisms.

Based on the MESUR group's consensus and the task group's agreement with it, the task group makes the following recommendations for control of forward contamination, each tied to specific mission objectives.

• **Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures.** Specific methods for sterilization are to be determined. Viking technology may be adequate, but requirements will undoubtedly be driven by the nature and sensitivity of the particular experiments. The objective of this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter and/or microorganisms deposited at the landing site.

• **Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures—such as clean-room assembly and cleaning of all components—for bioload reduction, but such spacecraft need not be sterilized.** Table 1.1 in Chapter 1 summarizes Viking-level procedures, and Appendix E includes a detailed description of the procedures.

The task group sees little utility in further attempts to estimate actual probability-of-contamination values in various martian environmental regimes. In the absence of crucial data relating to the survivability and growth potential of terrestrial organisms on Mars, such exercises are purely subjective. The task group emphasizes that the philosophical intent under-

lying the 1978 report—to protect Mars from terrestrial contamination so as
not to jeopardize future experiments aimed at detecting martian life—is still
profoundly important.

ADDITIONAL RECOMMENDATIONS

Recommendations for Research

**The task group strongly recommends that a sequence of unpiloted
missions to Mars be undertaken well in advance of a piloted mission.**
Any future changes in recommendations to ensure planetary protection, es-
pecially for piloted or sample return missions, will depend on the acquisi-
tion of new data. **With regard to these missions, the task group recom-
mends that a broad spectrum of martian sites be examined, with emphasis
on measurements that provide data most likely to contribute to models
that provide for a better understanding of the probability of life on
Mars and where best to go to find it.**

Until such data are available, it will be impossible to make informed
decisions concerning landings for in-depth biological study. Such data will
also greatly affect the ability to make future decisions concerning the rigor
required for spacecraft cleanliness and possible sterilization.

Location of martian lander sites should take into account our rudimen-
tary but growing understanding of Mars and our extensive knowledge of the
basic requirements of life. It is also important to consider the subsurface of
Mars. Within a site, it may prove important to plan for data collection that
probes below the readily accessible surface, in order to obtain information
on subsurface environments. Microenvironments—whether on the surface
or in isolated vents, cracks, or layers of the subsurface—may exist now or
may once have existed at some time in the past. Properly designed experi-
ments may be able to address the issue of spatial and (perhaps) temporal
heterogeneity and its possible relationship to our ability to evaluate the
biotic and abiotic status of a given site.

Collection of appropriate data should allow the scientific community to
amend planetary protection policy recommendations for back contamina-
tion, perhaps resulting in recommendations similar to the alterations in pro-
cedures for assessing forward contamination suggested by this task group.
The determination of current or inferred past geophysical conditions on Mars
may help identify locations where life-detection missions should be sent.

Recommendations Regarding Assessment of Spacecraft Bioload

The task group's recommendation to reduce bioload on all spacecraft and
to sterilize those spacecraft used in life-detection missions assumes the use

of Viking procedures. However, **the task group recommends that the Viking protocols for assessment of spacecraft bioloads be upgraded to include state-of-the-art methods for the determination of bioload.** It is critical that methods for assessing bioload be compatible with methods used to detect life, with methods for both assessment and detection reflecting the same limits and sensitivity. Data on bioloads of Viking components and spacecraft are not relevant to current life-detection procedures. Modern methods of bioburden assessment should be developed for and applied to spacecraft destined for future Mars missions, especially those carrying in situ extant life-detection experiments. Although immediate use of these techniques is not a feasible goal, the development of the methodology in anticipation of future life-detection missions is absolutely essential.

Recommendations Concerning Other Issues

Piloted Versus Unpiloted Missions

Missions carrying humans to Mars will contaminate the planet. It is therefore critical that every attempt be made to obtain evidence of past and/ or present life on Mars well before these missions occur. The issues of forward and back contamination have societal, legal, and international implications. These implications are serious, and they deserve discussion and attention.

Societal Issues

A substantial number of active national and international organizations are on the alert for environmental abuse. There is every reason to take seriously the concern (already expressed in some cases) about contamination of Mars and almost certainly about the issue of back contamination of Earth by martian samples. Although public concern over such issues is often sincere and productive, it at times becomes distorted and exaggerated in the media, leading to public misunderstanding and opposition. **The task group recommends that NASA inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return.**

Legal Issues

There are also legal issues that must be addressed, involving international restrictions as well as federal, state, and local statutes that may come into play. There are currently no binding international agreements concerning forward or back contamination. **The task group recommends as es-**

sential that efforts be made (1) to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and (2) to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars. Furthermore, the task group recommends that NASA make a strong effort to obtain international agreement for a planetary protection policy.

NASA Planetary Protection Program

Although a planetary protection officer currently exists at NASA, there is no budgeted program (as there was during the Viking Program) to implement needed planetary protection research, a public education program, examination of legal and international issues, and the like. The task group recommends that NASA redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the recommendations made in this report.

SUMMARY OF RECOMMENDATIONS

All of the recommendations put forward by the task group in this report are summarized below. Each is discussed further in the full report in the chapter(s) indicated.

1. Efforts should be made to adopt current molecular analytical methods for use in bioburden assessment and inventory procedures for spacecraft assembly and launch for future missions, and also to develop new methods for the same purposes (Chapters 4 and 5).

2. Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined; Viking technology may be adequate, but requirements will undoubtedly be driven by the nature and sensitivity of the particular experiments. The rationale for this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter that is deposited at the site by microorganisms or organic residues carried on the spacecraft (Chapter 5).

3. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures—such as clean-room assembly and cleaning of all components—for bioload reduction, but such spacecraft need not be sterilized (Chapter 5).

4. A sequence of unpiloted missions to Mars should be undertaken well in advance of a piloted mission (Chapter 6).

5. A broad spectrum of martian sites should be examined with emphasis on measurements that provide data most likely to contribute to a better

understanding of the probability of life on Mars and where best to go to be able to detect it (Chapter 6).

6. The Viking protocols for assessment of spacecraft bioloads should be upgraded to include state-of-the-art methods for the determination of bioload (Chapter 6).

7. NASA should inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return (Chapter 6).

8. It is essential to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars (Chapter 6).

9. NASA should make a strong effort to obtain international agreement for a planetary protection policy (Chapter 6).

10. NASA should redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the above recommendations (Chapter 6).

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13. See Space Science Board, National Research Council, 1978.

14. See DeVincenzi and Stabekis, 1984.

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1

Introduction

Understanding the origin and evolution of life has been an important
goal of NASA and continues to generate some of the more interesting scientific questions for all mankind: Where did we come from? When did life
begin? Does life exist elsewhere? If it exists elsewhere, is life similar to
that found on Earth? Although there are many theories regarding these
issues, there are as yet no definitive answers.

One promising approach to understanding the origin and evolution of life
is to search for life elsewhere, primarily on other planets, where physical,
hydrological, and geochemical properties might favor (or might have favored in the past) the existence of replicating biotic systems like those
found on Earth. If life or evidence of it is found elsewhere, then our views
of the evolution of life on Earth may change drastically, and our understanding of life processes and the cosmos will be enhanced dramatically.
Although the search for life and/or the chemical precursors of life can be
justified in many places in the cosmos, some areas appear more likely than
others to yield positive results. As articulated in *The Search for Life's
Origins*, a 1990 report of the Committee on Planetary Biology and Chemical Evolution of the National Research Council,[1] "Mars continues to be the
extraterrestrial body that holds greatest promise of scientific return on fundamental questions about the origin of life" (p. 71). While the committee
agreed that present evidence indicates that extant life on the surface of Mars
is not likely, it also stated that "there are reasonable prospects that evidence
of chemical evolution and fossil life might be found" (p. 71). Because of
these possibilities, the committee recommended that a major objective of
future research be "[t]o assess the isotopic, molecular, morphological, and

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environmental evidence for chemical evolution and the origin of life on Mars" (p. 71).

Mars has been the object of intense scientific scrutiny since the invention of the telescope. It has also been the object of intense speculation concerning the possible presence of life on that planet, a possibility that has often enjoyed considerable popular appeal. With the technological advances that accompanied the development of spacecraft, our ability to conduct detailed studies of the planets in the solar system improved dramatically.

However, as our knowledge of conditions on the surface of Mars has increased, there has been a concomitant decrease in any expectation that life as we know it could exist on the planet. The Mariner spacecraft, which made both flyby and orbiter measurements, and later the Viking orbiters and landers, provided much new information about the chemical and physical nature of Mars. Viking attempted to look directly for life and for organic molecules commonly associated with life at two landing sites on the surface. No organic matter was found, and most scientists agree that no indications of life were detected. Granting these observations, it is also quite clear, however, that (1) the Viking experiments were performed at only two sites, which may not have been representative of the whole planet, and (2) the early state of Mars seems to have been very different from its present state and may have been characterized by the presence of abundant liquid water and a more substantial atmosphere.[2-4] Given these considerations, the search for life on Mars must include examination of other, more desirable sites (e.g., those where water has been present in the past) where life or a fossil (organismal and/or chemical) record may possibly exist.

The possibility that evidence of chemical evolution and/or fossil life might be found on Mars has led many scientists to embrace the conclusion, expressed in *The Search for Life's Origins*, that continued chemical, physical, environmental, and biological study of Mars is a scientifically sound enterprise. It is thus not surprising that scientists from many different nations are planning to participate in missions to Mars to investigate its properties by using a variety of different approaches, including remote sensing, use of surface landers, sample return, and eventually piloted exploration. Some of these missions are planned to occur within the next few years, including missions that involve landing experimental modules and penetrators on the martian surface.

Assuming that Mars will be further investigated, it is imperative that precautions be taken to ensure planetary protection, including protection from both forward and back contamination. The problem of forward contamination includes (1) the invasion of martian ecosystems by organisms from Earth that would be capable of growing and prospering, and (2) contamination by terrestrial biological material that would then be measured by our life-detection experiments. The latter is of great concern as it would

compromise a major part of the scientific rationale for the biological study of Mars.

The problem of back contamination concerns the possible return of potentially harmful biota to Earth. This issue is driven by many factors—societal, political, legal, ethical, and others—in addition to purely scientific concerns. Back contamination must be given the most serious and careful consideration in missions where samples are to be returned to Earth for analysis, and in piloted missions. To a large extent, the amounts and types of measures needed for protection against back contamination will be established on the basis of data gathered from upcoming missions now in their planning stages. That is, if it is established that life does not exist and has not existed on Mars, then the need for protection of Earth-bound samples will be obviated. On the other hand, if there is a suspicion of extant or past life, then the need for protection will have to be adjusted accordingly.

Historically, NASA's interpretation of planetary protection and its implementation of related procedures have focused on specific concerns related to forward and back contamination. First, in the face of possible forward contamination, the concern has been to preserve conditions on the planets for the future conduct of experiments on biological and organic constituents that might lead to insights concerning the origin and evolution of life in the cosmos. Despite other issues such as the dispersal and survival of species, the major focus has been on preserving other planetary environments from contamination by organisms from Earth that might grow there and thus obscure **forever** any efforts to understand the origin and evolution of life at locations other than Earth. Central to this issue is an assessment of the probability that an earthbound organism could contaminate another planetary body. Contamination in this case includes not only delivery of viable organisms, but also the growth of such organisms on the planet to such an extent that they would compromise future scientific endeavors.

The ability to estimate the probability of contamination (P_c) depends on two factors: (1) accurate knowledge of the limits of organisms' ability to survive and grow on Earth and (2) accurate knowledge of the surface conditions of the planet to be visited. Any constraints imposed on a mission to ensure planetary protection from forward contamination will be mission dependent, relying on the best possible information about the conditions that might support growth of any biota from Earth that will survive transit through space. These points are extremely important; as information accumulates about a given extraterrestrial body, assessment of the amount of planetary protection needed to prevent contamination will undoubtedly change accordingly. The process must be iterative and must allow for modifying the techniques to ensure protection as new information is acquired regarding the harshness of the planet and the probability of contamination.

It should be obvious that if life is to be detected on Mars (a possibility,

TABLE 1.1   Viking-Level Procedures Recommended for Future Mars Missions

Mission Type and Objective	Procedure
1. Landers with in situ extant life-detection experiments	Trajectory biasing and orbit lifetime Cleaning of components[a] Clean-room assembly Surface cleaning Lander sterilization Protection from recontamination Bioburden assessment
2. Orbiters and landers without in situ extant life-detection experiments	Trajectory biasing and orbit lifetime Cleaning of components[a] Clean-room assembly Surface cleaning Bioburden assessment

[a]Levels and types of cleaning will depend on the particular measurements being performed during the mission. For example, the Viking landing craft were cleaned to remove organics to less than 1 ng cm^{-2} because they were measuring organic molecules. This removal of organic material was accomplished via detergent cleaning, solvent cleaning, and hot helium purges to remove solvents.[5] Similar levels of cleaning should be sought in future extant life-detection studies, but these will undoubtedly be modified upwards in landers that have no in situ extant life-detection experiments.

given the remarkable sensitivity of modern techniques and approaches), great care must be taken not to compromise such scientific goals by a previous or simultaneous introduction of life forms from Earth. Rigorous precautions were taken during the Viking mission to ensure that no forward contamination occurred. However, substantial amounts of data have accumulated since that time. Thus it is quite possible that the recommendations for planetary protection that guided the Viking mission may not be suitable for missions being flown today or for those flown in the future. An outline of Viking sterilization procedures is shown in Table 1.1, and a more detailed explanation is included in Appendix E of this report.

The second focus of NASA's planetary protection effort has been, and will continue to be, protection of Earth's biosphere from the possibility of back contamination by any forms of life that may exist on other planets or bodies that might be visited. Both in missions where samples are to be returned to Earth for analysis and in piloted missions returning both samples and crew, a variety of scientific, societal, and legal reasons exist for a planetary protection policy that ensures (1) the integrity and safety of our planet and (2) the rigorous protection of the scientific integrity of the samples. The two goals should be accomplished with a common protocol.

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2

Summary of Planned Future Missions

The following is a brief summary of upcoming or planned missions to Mars. The only approved missions are the U.S. Mars Observer mission to be launched in October 1992 and the Soviet Mars 1994/96 mission. Both NASA and ESA are studying a network mission that involves placing numerous small stations on the surface of the planet. In addition, both the United States and the former Soviets have been studying various sample return missions that may also involve the use of rovers.

APPROVED MISSIONS

U.S. Mars Observer Mission

Mars Observer will arrive at the planet in September 1993. After a checkout period, the spacecraft will be placed in a high-inclination mapping orbit and will start to systematically observe the planet.[1] The mapping orbit is such that the spacecraft will have less than 1 chance in 10^{-4} of impacting the planet before 2038. The spacecraft will have a variety of instruments directed at characterizing both the surface and the atmosphere. An altimeter will determine surface elevations to a vertical precision of a few meters, and the surface will be imaged at a resolution of roughly 100 meters per pixel. A magnetometer will determine if the planet has an intrinsic magnetic field, map any crustal remnant field, and follow variations in the magnetic field induced by the solar wind or surface anomalies. The surface chemistry and mineralogy will be mapped by two instruments: (1) a gamma-ray spectrometer will determine all major elements and most minor ele-

ments with spatial resolution of roughly 300 kilometers per pixel, while (2) a thermal emission spectrometer will map variations in surface mineralogy at a spatial resolution of roughly 3 kilometers per pixel. An on-board camera will be used to assess daily variations in cloud patterns, as well as to image small areas of the surface at a resolution of 1.5 meters per pixel. A pressure modulated infrared radiometer will repeatedly sound the atmosphere to characterize changes in the vertical structure of the atmosphere with time and location. Finally, on-board transponders will permit extremely precise determination of the planet's gravitational field. The nominal mission will last for 1 Mars year, or roughly 2 Earth years. The spacecraft has a relay antenna designed to receive data from Soviet surface stations to be launched in 1994 and 1996; after completion of the nominal mission, the spacecraft will be used in part to support these surface stations. It is also expected to continue to make observations of the planet, perhaps focusing on areas of special interest identified during the nominal mission.

Soviet Mars 94/96 Mission

The former Soviets are planning to launch a spacecraft to Mars in 1994.[2] It will be primarily an orbiter instrumented to make a variety of observations of the surface, atmosphere, and ionosphere. Among these instruments, those of greatest biological interest are an imaging system that will image large regions of the planet at a resolution of 10 meters per pixel, a near-infrared imaging spectrometer for determination of surface mineralogy, and ground-penetrating radar that could detect anomalies caused by the presence of water near the surface. Of concern from the point of view of planetary protection are stations that will land on the surface. The spacecraft will carry two penetrators designed to be released from the spacecraft 3 to 4 days before arrival at Mars. The penetrators will separate on impact with the ground. The forebody will penetrate the ground to a depth on the order of meters, while the aft body will remain resting on the surface still wired to the forebody. Within the forebody will be various analytical instruments such as a gamma-ray spectrometer and a seismometer. The aft body will have an array of meteorological instruments, a camera, and a transmitter. The parent spacecraft will also release two small stations 3 to 4 days before arrival at Mars. These stations will land on the surface and deploy an array of instruments similar to those in the penetrators. Both the penetrators and the small stations are planned to last for 1 Mars year. The landing sites are restricted to latitudes from about 20°S to 60°N.

In 1996, the former Soviets plan to launch a second spacecraft similar to that launched in 1994, except that it will place in Mars orbit a module from which will be launched a balloon and small rover.[3] Both the balloon and the rover will be released to the surface simultaneously and are expected to

land in the same part of the planet. The preferred landing sites are at high latitudes (50 to 60°N) for reasons of balloon safety. The balloon will be 30 meters high, constructed of 6-micron mylar, and designed to land at night and float during the day. It is expected to last for as long as 10 days, during which it could travel as far as 1000 kilometers. During the night it will drag an instrumented guide rope along the ground. The gondola of the balloon will carry a camera and various instruments to measure the chemistry and mineralogy of the soil, as well as any water present, and to monitor the atmosphere and magnetic field. One concern is that it will be difficult to reduce the bioload because of the balloon's fragility and size, and there is no information available on what decontamination procedures will be used. Since the balloon will move large distances dragging a guide rope and instruments along the ground, the potential for contamination is significant.

The Mars 96 rover will weigh roughly 100 kilograms and be about a meter in height.[4] In addition to imaging instruments, it will carry instruments to measure soil mineralogy and chemistry, the water content of the soil, and trace gases in the atmosphere, and it may include capabilities for analyzing organic materials. It will have a drill that can bring to the surface, for analysis, material from a depth of as much as 2 meters below the surface. The lifetime is nominally 1 Mars year. The distance that it can travel in this time will depend on the terrain it encounters, but could be as much as several hundred kilometers.

CONTEMPLATED MISSIONS

U.S. MESUR Mission

The United States has been studying the feasibility of placing a network of simultaneously operating stations on the martian surface.[5] The objectives of the network are (1) to determine the chemistry and mineralogy of martian soils and rocks at different locations representative of martian heterogeneity, (2) to observe the fine-scale structure of the surface in different geologic environments, (3) to determine the seismicity and internal structure of the planet, and (4) to improve our understanding of the circulation of the atmosphere and the structure of the boundary layer. The network will be built by launching four to eight small (1.3-meter diameter), relatively inexpensive spacecraft on successive launch opportunities spanning a 4-year period, possibly starting in the late 1990s. The plan is that 16 stations will be operating simultaneously on the surface at the end of the launch period and that they will survive for 1 full Mars year after all are in place. The MESUR mission will thus have a total lifetime of about 7 Earth years. The stations will fly independently to Mars and will have the ability to land

almost anywhere on the planet. Each will carry a seismometer, a camera, instruments for determination of the chemistry and mineralogy of rocks and soil, and a meteorology package. The mode of instrument deployment and whether the subsurface can be accessed are aspects still being studied. The stations will be widely distributed, and some will be sent to places such as the poles that are unlikely to be visited by other types of landers in the foreseeable future.

ESA Marsnet Mission

The European Space Agency has been independently studying a network mission called Marsnet.[6] The design is very similar to that of the MESUR mission except that fewer stations are involved, and the mechanism for delivery of the stations to the martian surface is uncertain. The array of instruments proposed for Marsnet is similar to that proposed for MESUR. Preliminary discussions have been undertaken to determine how the two concepts might be merged.

Sample Return and Rover Missions

The return of samples from Mars has had high scientific priority but has been deferred in favor of other missions because of its high cost. A U.S. sample return mission before 2000 is extremely unlikely, but the former Soviets have at times suggested that they would like to launch such a mission by that year. There are many ways to implement a sample return mission. In the late 1980s, several types of sample return missions were studied. They involved the return of 5 to 10 kilograms of sample and the use of large (1000-kilogram) rovers to collect and document samples from many different locations. Such missions would be so expensive that conducting them would require major changes in the way planetary science is funded.

More recently, simpler sample return techniques have been studied.[7] These techniques take advantage of the miniaturization of spacecraft components and analytical instruments, as well as the reduced amount of sample that is required by modern analytical techniques. The general philosophy is to send small sample return missions to several locations to obtain a variety of samples, rather than relying on an elaborate rover to provide a range of samples. The missions could still carry rovers to acquire samples, but the rovers might weigh on the order of 10 kilograms rather than 1000 kilograms. These approaches reduce the projected cost of sample return missions by a factor of 10. Which, if any, of these missions will actually fly is uncertain in light of the current worldwide economic situation.

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3

Surface Environment of Mars

Despite an incomplete understanding of the Mars surface environment, it is generally agreed that conditions are extremely inhospitable to life from Earth. This chapter reviews various aspects of the surface environment, focusing on several that may have relevance to the issue of forward contamination, including both the growth of organisms from Earth on Mars and the lifetime of bioorganic matter deposited on the martian surface.

SURFACE CHEMISTRY

Our understanding of the chemistry and mineralogy of the martian surface is incomplete and is based primarily on (1) the Viking lander experiments; (2) evidence from the shergottite, nakhlite, and chassignite (SNC) meteorites; and (3) remote sensing data.[1-4] Results from the gas chromatograph-mass spectrometer (GC-MS) have indicated the presence of 0.1 to 1.0 percent bound water in the soil. The Viking lander inorganic experiment detected most major elements heavier than magnesium and a number of minor elements. Several chemical species of biologic significance (C, N, H_2O, P) were left undetermined and had to be inferred. Several analyses were obtained at each of two sites, and the remarkable similarity in composition of all the materials suggested that the material had been homogenized over the whole planet by repeated dust storms. Viking carried no mineralogy experiment, and so mineralogy had to be inferred. Two competing models for the mineralogy of the soil are that it (1) consists largely of iron-rich clays or (2) resembles an amorphous, partly hydrated volcanic ash called palagonite. Although faint traces of secondary minerals, such as

carbonates, have been detected by spectroscopic analyses, the general lack of absorption features suggests that the soil is poorly crystalline.

Part of our current understanding of Mars comes from the analyses of the SNC meteorites,[5,6] which are composed of basalts that have crystallized from melts within the past 1.3 billion years. These meteorites were originally suspected to be of martian origin because there was no other plausible parent body that could have erupted basalts so recently. A martian origin appears to have been confirmed from analyses of gases trapped within the meteorites. The isotopic ratios of nitrogen, argon, and xenon are identical, within analytical error, to the ratios found in the martian atmosphere, as determined by Viking, and are distinctively different from those of any other known source in the solar system, including Earth. The SNC meteorites contain a variety of secondary minerals such as illite and smectite clays and water-precipitated salts such as calcium and magnesium carbonate, calcium sulfate, magnesium phosphate, and hematite (Table 3.1). Migration of these water soluble salts within the soil is suggested by the presence of cemented soil at the Viking sites. The chemistry of the soil as determined at the Viking sites is consistent with the mixture of the minerals found in the SNC meteorites, possibly with the addition of palagonite. Because of the mafic nature of the soil, and the basaltic composition of the SNC meteorites, the dominant rocks exposed at the surface are thought to be basaltic.

TABLE 3.1 Mars Biogeochemistry from SNC Meteorites

Water-Precipitated Minerals Confirmed	Shergottite EETA79001	Nakhla	Chassigny
$CaCO_3$	X	X	X
Mg-bearing $CaCO_3$	X		
$MgCO_3$			X
$(Fe,Mn)CO_3$		X	
$CaSO_4 \cdot nH_2O$	X	X	X
$(Mg)_x(PO_4)_y \cdot nH_2O$	X		
$(Mg)_x(SO_4)_y \cdot nH_2O$		X	
$(Na,K)Cl$		X	
"Illite" $(K,Na,Ca_{0.5},H_3O)(Al,Mg,Fe)_2$ $O_{10}[(OH)_2,H_2O]$	X		
S,Cl-bearing micabole	X		
Smectite $(Na,Ca_{0.5})_{0.3}(Al,Mg,Fe)_{2-3}$ $(Si,Al)_4O_{10}(OH)_2 \cdot nH_2O$		X	
$Fe_2O_3 \cdot nH_2O$		X	

SOURCE: James L. Gooding, Johnson Space Center, NASA.

The chemistry of the soil is of particular biologic interest. One of the major surprises of the Viking missions was the failure of the GC-MS to detect organics in samples to depths of about 10 centimeters, despite the expectation of finding at least some organics of meteoritic origin. The soils were also found to be oxidizing: 70 to 800 nanomoles of O_2 were released upon humidification of the soil, and nutrients added to the soil were oxidized. Although the exact nature of the oxidants is unknown, they probably form as a result of (1) condensation on the surface of OH, HO_2, and superoxides formed by ultraviolet (UV)-induced photolysis of water in the atmosphere and/or (2) UV-induced photolysis of water absorbed on soil particles.[7] The depth to which the soil is oxidizing and is devoid of organics is not known, but much of the loose material near the surface is likely to be episodically turned over, exposed to the surface, and blown around the planet as a result of wind action. The expectation is, therefore, that the Viking results are applicable, in general, to loose, wind-deposited materials at the surface.

ULTRAVIOLET AND IONIZING RADIATION

Although on Mars no radiation with a wavelength of less than 1900 angstroms (Å) reaches the surface because of strong adsorption by CO_2, in comparison to Earth the martian surface is only minimally shielded from longer-wavelength UV radiation.[8] On Earth a deep ozone absorption band at 2550 Å prevents most UV from reaching the surface. In contrast, ozone is present only at high latitudes in the martian winter hemisphere and in amounts typically in the range of 30 to 60 micrometer-atmospheres, an amount much smaller than that shielding Earth. These amounts of ozone on Mars can attenuate the UV to 10^{-3} as compared with 10^{-30} for Earth. At low latitudes and during the summer at high latitudes, there is essentially no attenuation, and the full solar flux at wavelengths greater than 1900 Å falls on the martian surface unless attenuated by aerosols in the atmosphere. Significant reduction by scattering is expected only in the dust storm season, which lasts roughly one-quarter of the year. Thus, during the entire martian year, the UV flux is sufficient to sterilize the surface environment.

Mars is less protected than Earth from ionizing radiation because Mars has no magnetic field and only a thin atmosphere. The main concern is with galactic cosmic rays (GCRs) and occasional solar flare particle fluxes. GCR heavy ions, although significantly less abundant than GCR protons, contribute most of the annual biological dose-equivalent of GCRs at the martian surface. Doses from secondary radiation also accrue within the upper 50 centimeters of the regolith. At low elevations, where the atmosphere provides maximum protection, the GCR doses approach annual limits allowed for humans but fall far short of values commonly certified for

sterilization of food. Ionizing radiation does not appear, therefore, to be sterilizing for the short term, although the effects of such exposures over many years are unclear.

TEMPERATURE

Temperatures are of particular biological interest because of their influence on the stability of water. Surface temperatures are determined mainly by latitude and season and by the properties of the surface, especially thermal inertia and albedo.[9] Mean daily temperatures range from 215 K at the equator to 150 K at the poles. Daily excursions from the mean are controlled largely by the thermal inertia of the soil. Martian soils have very low thermal inertias compared with those of typical terrestrial soils, which, together with the thin atmosphere, cause the near surface to heat rapidly during the day and cool rapidly at night. As a result, equatorial temperatures can range from as low as 180 K at night to 290 K at noon. However, these daily fluctuations damp out rapidly at depth, such that at a few centimeters depth the temperatures remain close to the diurnal mean of 215 K. Temperatures at the poles remain close to 150 K, the condensation temperature of CO_2, for most of the year. For a short period in midsummer, CO_2 completely sublimes at the north pole, exposing a water-ice cap and allowing the surface temperature to rise to 200 to 210 K on the water-ice and possibly to 230 K on dark ground. At the south pole, only incomplete sublimation of the CO_2 was observed during the year that Viking viewed Mars; even then, however, within the seasonal cap some ground was exposed, which rose to higher temperatures. Because the CO_2 cap disappears for only a short period of time, the mean annual temperature at both poles is close to 150 K, and at depths greater than 1 to 2 meters, the ground remains permanently at this temperature. Any summer increase in ground temperature is restricted to shallower depths.

WATER

Estimates of the amount of water present at the martian surface have ranged widely in recent years.[10-13] However, recent recognition of the efficacy of gas-dynamic escape and impact erosion in removing volatiles from the planet early in its history has undermined geochemical arguments for low water abundances and has led to greater credence of the higher geologic estimates based on the observed effects of water on the surface. Recent estimates suggest that if all the water that flowed across the surface during the last 3.8 billion years were spread evenly over the planet, it would form a layer tens to hundreds of meters deep. For comparison, all the water present at the surface of Earth would form a layer 2.7 kilometers deep. The total

crustal inventory of water on Mars is difficult to assess, but it could be considerably larger than that which flowed across the surface. Identifiable near-surface reservoirs are the residual north polar ice cap, the polar layered terrains, and water absorbed in the regolith minerals. All these reservoirs are probably small in comparison to the total inventory. Most of the water is thought to occur as ground ice and, at depths greater than 1 kilometer, as ground water. The atmosphere contains very little water (10^{-3} M), but it is close to saturation for nighttime conditions.

For the average amount of water present in the atmosphere of 10 precipitable microns of water, the frost-point temperature is 200 K (corresponding to a vapor pressure of water of 0.1 pascal). Any part of the near surface of the planet where the temperature exceeds 200 K should be ice-free because of the slow sublimation of the ice over geologic time. At low latitudes, where mean annual temperatures exceed 200 K, the ground is generally expected to be ice-free to depths of a few hundred meters. However, anomalous combinations of albedo, thermal inertia, and porosity could result in near-surface ice locally.

At latitudes in excess of 30 to 40°, ice may be present at depths greater than 1 to 2 meters, the depth of penetration of the annual wave, because mean annual temperatures are below 200 K. At shallow depths small amounts of ice could be present only transiently as water vapor moves in and out of the soil in response to the seasonal temperature cycle. Although there are no direct measurements of ground ice at these high latitudes, there is abundant geologic evidence that, in contrast to low latitudes, ice is indeed present.

The stability conditions just described are equilibrium conditions, and various events could result in the presence of ice in disequilibrium. If ice were buried beneath a few centimeters in equatorial soil, sublimation rates would be very low (about 10^{-5} gm cm^{-2} yr^{-1}). If water were supplied at a rate greater than this, such as by volcanic action, then ice could accumulate near the surface, despite being in disequilibrium with the atmosphere.

VOLCANISM

Volcanism is of biologic interest because of the possibility of hydrothermal activity and because of its potential effects on the distribution of water. Evidence from both counts of impact craters and chemical analyses of SNC meteorites suggest that Mars has been volcanically active in the recent past and thus could still be volcanically active today. Crater counts suggest that parts of the surface could be as young as 10^8 years, a number consistent with the estimated ages of the SNC meteorites, which suggest that volcanic activity could have occurred as recently as 10^8 years ago; geologically this is very recent. However, even if the planet is currently volcanically active, the rates of volcanic activity must be orders of magnitude lower than those

found on Earth, because young surfaces are so restricted in area. There is no direct evidence of current volcanic activity, such as thermal anomalies or volcanism, which might be accompanied by hydrothermal activity. Sites of such activity may be identified by venting of steam and/or local concentrations of hydrothermal minerals. Alternatively, all of the hydrothermal activity might be associated with subsurface environments without any surface manifestations.

FORMER CLIMATIC CONDITIONS ON MARS

Although present-day Mars is very hostile to life, there are good reasons to believe that Mars has experienced more hospitable conditions in the past. The evidence is particularly strong for the very early history of the planet, during the times that life first started on Earth. For most of Mars' history, erosion rates would have been extremely low. However, terrains that date from the early period are highly degraded and commonly dissected by branching valley networks.[14,15] The networks resemble dry terrestrial river valleys and are thought to have been formed by slow erosion owing to running water. Despite uncertainty about the precise conditions required for these valleys to form, it is probable that some combination of high heat flow and high surface temperatures is required. For small streams to flow any appreciable distance, the surface temperature must be at or above 0°C. To maintain such a temperature, an atmosphere of at least 1 to 2 bars of CO_2 was probably required. It has accordingly been suggested that about 3.5 billion to 3.8 billion years ago the surface of Mars, being warm and wet, was hospitable to life.[16] However, after this time most of the CO_2 was permanently removed to form carbonates, and the surface of the planet evolved to its present cold, dry conditions. If life started during the early era, it might have survived at least for a time, either intact or as biochemical remnants in isolated niches such as in hydrothermal systems, subsurface brines, or endolithic environments.

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4

Limits of Life on Earth: Expansion of the Microbial World and Detection of Life

The Task Group on Planetary Protection assessed past reports and current views on the range of environmental conditions believed to exist on Mars and reached the consensus that it is extremely unlikely that a terrestrial organism could grow on the surface of Mars, although survival for some time is possible. It is clear that the most extreme environments on Earth where organisms can replicate are still considerably less extreme in some parameters vital for life than are known to occur over most of the martian surface. Particularly important in this regard are the high levels of UV radiation, the thin atmosphere, the extremely low temperatures, and the absence of liquid water on the surface of Mars.

This appraisal is based on our current understanding of the conditions on Earth that limit cell growth; however, the task group emphasizes that although it is extremely unlikely that terrestrial organisms could grow on the surface of Mars, this does not imply that life does not exist anywhere on Mars. There is far too little information to assess the possibility that life may exist in subsurface environments associated with hydrothermal activity or in selected microenvironments free from the harsh conditions already mentioned, or to conclude that organisms resembling terrestrial life forms did not evolve on Mars during the planet's early geological history.

The primary residual concern of the task group is with forward contamination by intact cells or components of cells that could be detected by sophisticated molecular methods in future expeditions designed to look for evidence of extant or past life on Mars. The task group believes that this concern necessitates that those involved in the planning of present and future expeditions to Mars be appraised of new results obtained from

studies of extreme environments as well as the inevitable extension of the limits of environments where growth and survival can take place. Underscoring all of these advances in the microbiology of extreme environments are parallel advances in the development of new methods and more accurate and sensitive instruments for detecting the presence of life and life-related molecules and for identifying their evolutionary relatedness.

It is not a straightforward matter to define the ranges of physical and chemical conditions on Earth in which organisms can grow, replicate, or survive for extended periods. During the 13 years since the SSB's last report on planetary protection,[1] bacteria have been detected or isolated from many hostile environments on Earth, including the dry, extremely cold surfaces and interstices of rocks in the dry valleys of the Antarctic, hot environments associated with submarine and terrestrial volcanoes and geothermal systems, and deep subsurface sediments and aquifers. These investigations are in their infancy, and we still know little about either most of the organisms inhabiting these environments or in many cases the geochemistry and geophysics of the environments.

In the last decade or so, a variety of novel organisms have been isolated. They include hyperthermophiles capable of growing at 110°C, obligate barophiles capable of growing at the pressures found in the deepest ocean trenches, and anaerobes capable of using iron, manganese, or even uranium as electron acceptors. Similarly, a variety of strategies have been identified by which microorganisms can survive environmental conditions that do not allow growth, including low temperature and low nutrient conditions. Traditionally, endospore and cyst development were considered the principal mechanisms for long-term survival by microorganisms, but it is now clear that many microorganisms have mechanisms for long-term survival that do not involve spore or cyst formation. It is now recognized that the inability to culture many microorganisms is a widespread phenomenon apparent with environmental samples and that only a few percent (or less) of organisms detected by microscopic methods can usually be cultured. Examples of the manifestation of organismal survival mechanisms include both the miniaturization of cells and the attachment to surfaces.

EXTREME THERMOPHILES AND VOLCANIC ENVIRONMENTS

Important recent discoveries and hypotheses, published across a diversity of disciplines, have pointed to submarine hydrothermal vent systems, and specifically to their subsurface crustal environments, as the likely site of biochemical and even early biological evolution.[2] The particular nature of organisms that might have evolved on Mars is unknown. As to organisms that may have been transported to Mars from Earth during Archaean bom-

bardments, there is strong phylogenetic evidence, based on both 16S rRNA sequence comparisons of a large number of organisms and geological evidence, that the earliest groups of microorganisms to inhabit Earth were anaerobic hyperthermophiles (growth at 90°C or higher).[3,4] These organisms, which utilize carbon and energy sources found in hydrothermal and geothermal systems and possess unusual mechanisms for growth at temperatures exceeding 100°C, constitute a distinct phylogenetic group of organisms that share some characteristics with other bacteria and eucaryotes as well. Originally classified as a distinct kingdom, the archaebacteria are now classified in the domain Archaea and are more closely related to the domain of the Eucarya (formerly eucaryota) than to the Bacteria (formerly eubacteria).[5]

Hyperthermophiles are significant to a discussion of planetary protection issues because of evidence already presented that active volcanism may occur on Mars today. Implied is that hydrothermal activity would accompany volcanism because of water entrapped in the martian crust. Although the chance that a hyperthermophilic Archaea from Earth would contaminate Mars at a location that would allow growth is extremely remote, these organisms could be more significant with respect to back contamination, and as Earth analogues to past martian life if life ever existed on Mars.

Among the many unusual properties of hyperthermophilic Archaea, those properties important to concerns about planetary protection include the probability of survival and growth under any of the ranges of physical and chemical conditions that exist on Mars. Unfortunately, we know considerably less about the survival of hyperthermophilic Archaea than we know about Bacteria, spores, fungi, and viruses. Only recently have extremely thermostable enzymes from vent hyperthermophiles been purified and characterized. For example, an amylase from *Pyrococcus furiosus*—a heterotroph capable of growing at temperatures up to 103°C—has a half-life of 2 hours in an autoclave at 120°C and is active at 140°C.[6] A purified α-glucosidase, with a half-life of 48 hours at 98°C, reaches optimal activity in the temperature range of 105 to 115°C.[7] The other extremely thermal stable enzymes studied from hyperthermophiles include ferredoxin, hydrogenase, serine protease, glyceraldehyde-3-phosphate dehydrogenase, and a never-before-described tungsten-iron-sulfur enzyme from *P. furiosus* that catalyzes a dehydrogenase-like reaction of very low potential at 100°C.[8] Besides proteins, other macromolecules from hyperthermophiles, including DNA and membrane lipids, must also have some unusual properties. Recently, the presence of a reverse gyrase, which catalyzes positive supercoiling of circular DNA, was discovered in all hyperthermophiles tested.[9] It was suggested that supercoiling of DNA imparts thermostability.

Questions regarding thermal stability of Archaea cells and their macromolecules and synthetic systems have only recently been addressed. Preliminary results, however, point to unique structures and mechanisms for

growth and survival under some of the more extreme conditions on Earth, although these conditions are not nearly so severe as surface conditions on Mars.

LIFE IN EXTREME ENVIRONMENTS

Dormant Forms of Life

Endospores from the gram-positive bacteria are ubiquitous and perhaps the most resistant and survivable form of life on Earth. They are known to survive for thousands of years and are resistant to freezing, desiccation, and vacuum and are highly resistant to many disinfectants. Bacterial spores are also moderately resistant to heat and to UV and ionizing radiation. Some spores germinate whenever there is free water, ranging in temperature from subfreezing to superboiling. Recognition of the ability of spores to survive such harsh conditions has led previous committees to focus on bacterial endospores as a major concern in planetary protection.

Recent studies utilizing the NASA Long Duration Exposure Facility (LDEF) have shown good survival of multilayers of bacterial spores, which had been fortified with buffer and nutrients, after 6 years of exposure to space vacuum.[10] When spores were not shielded from solar radiation, their survival was reduced to 10^{-2} to 10^{-4} percent. These results suggest that the vacuum and cold conditions of space pose no particular barriers for spore survival, but in the absence of shielding from UV radiation, there is little chance for the survival of dormant spores transported through the space environment. Other studies have focused on comparisons of the survival rates of spores exposed to UV irradiation under atmospheric and vacuum conditions, and at a variety of temperatures.[11,12] Conditions simulating interstellar space or those of the surface of Mars inactivate spores rather quickly, suggesting that any long-term survival of unshielded spores on Mars would be impossible. It has not been possible to specify the mechanism of spore inactivation, although it appears that spore photoproducts, such as thymine dimers, are not responsible.[13] On the basis of such laboratory experiments, it has been proposed that with proper shielding, bacterial spores might survive UV irradiation for very long periods, perhaps millions of years.[14]

Deep-Subsurface Microbes

Experiments since 1984 supported by the Department of Energy (DOE) have allowed the recovery of viable bacteria from subsurface sediments 500 to 700 meters below Earth's surface.[15] In these experiments, there is clear evidence that the organisms recovered from the deep subsurface are not contaminants from the surface, from more superficial sedimentary horizons,

or from the drilling fluids. These organisms are found in Middendorf Cretaceous sediments, which are 100 million years old and form an aquifer in which the time for ground water recharge is at least 15,000 to 20,000. The ground water from the bore holes contains traces of refractory carbon and about 2 milligrams of oxygen per liter. For the oxygen to be maintained in the face of a living microbiological community of 10^6 microbes per cubic centimeter, the growth rate in terms of the doubling time of these organisms must be between 10,000 and 20,000 years.[16] Based on analysis of total 16 S rRNA sequences from these organisms, it is known that they constitute primarily a subset of unique *Pseudomonas* and *Arthrobacter* species. They clearly have the capacity to exist in a viable but dormant state for very long periods of time. These organisms appear to have a highly developed capacity to repair their DNA as evidenced by their very high resistance to UV radiation. The ability of Earth microbes with a full complement of enzymes to exist in relatively suspended animation for extended periods, yet to be ready for instant growth, has direct implications for planetary protection requirements related to forward contamination as well as sample return. The ability to maintain efficient DNA repair in the absence of cell division (which these organisms are apparently able to do) is a property that may be of great advantage for long-term survival in space and on Mars.

Extreme Halophiles

A preliminary (and as yet unpublished) report involving halophilic bacteria was presented to the task group and deserves at least a passing mention here. Microorganisms embedded in crystals of salt have recently been identified at the DOE Waste Isolation Pilot Project high-intensity storage site in New Mexico. The salt deposits have been dated as being approximately 200 million years old. Extremely halophilic bacteria, of the domain Archaea, have been cultured from the interior of the salt crystals, which suggests the possibility that bacteria have remained viable within the salt crystals. Such a potential for extended survival and the capacity of these organisms for growth in brines, which may be present in the martian subsurface, make this group of extreme halophiles very interesting with regard to possible types of organisms to look for in the search for extant or past life on Mars. With regard to forward contamination, the possibility that such an organism could reach a suitable environment, even if it survived the trip through space, seems vanishingly small.

Cryptoendoliths

Some microorganisms in the Antarctic have adapted to extremes of low temperature, high winds, and lack of water by forming communities within

sandstone. Inside the rock, an increased relative humidity provides adequate water for growth, and light penetration is adequate for photosynthesis for very short periods that occur no more often than 2 to 5 days per year.[17] For example, in the Dry Valley region of Antarctica, a microbial ecosystem exists in the interstices of porous sandstone, complete with primary producers (lichen algae, green algae, and cyanobacteria) and primary microbial consumers (decomposers such as yeast, bacteria, and filamentous fungi), but lacking higher trophic-level consumers.[18,19] This microbial community has apparently adapted to life in these rocks to avoid the harsh external conditions, which include (1) high-velocity winds, (2) low temperatures (the rocks warm to above-freezing temperatures due to their low albedo and high thermal inertia), (3) low moisture (the rocks retain water from snow melt), and (4) high UV flux on the surface. Conditions of light, temperature, and water that permit slow metabolic rates occur only rarely, perhaps for about 100 to 200 hours per year, and rates of cellular metabolism and growth in these communities are perhaps the lowest found on Earth. (For example, the carbon turnover, a reflection of metabolic rate, has been estimated to be on the order of 10,000 to over 20,000 years.[20] All the inorganic nutrients needed for growth come from the minerals in the rock matrix and thus are not limiting to the community.[21] The community can carry out photosynthetic metabolism at temperatures as low as $-8°C$. Cryptoendolithic lipids, which can stay fluid to $-20°C$, may be important for the organisms to metabolize in such cold conditions.[22] Clearly these microbes have adapted to harsh environmental conditions, and these communities may provide reasonable models for survival strategies that might be adopted by microbes as conditions change from above-freezing temperatures and flowing water to temperatures below $0°C$ and limited free water.

Barophiles

If liquid water is present at kilometer depths on Mars, microbial life in that environment may face environmental conditions similar to those experienced by the barophilic bacteria isolated from the deep sea on Earth. Pressure is a significant factor in the growth of the barophilic bacteria, with the optimal pressure for growth being similar to the pressures found in the deep-sea regions from which the bacteria were isolated.[23] Studies of these bacteria will help to establish the limits of pressure that can be tolerated by life on Earth and will guide future life-detection experiments conducted beneath the surface of Mars. For instance, is it possible that liquid water exists at depth (due to geothermal heating) and supports a community of barophilic organisms similar to those described for Earth organisms?

Radiation-Resistant Bacteria

There is a wide range of microbial sensitivity to radiation stress induced by either UV or ionizing radiation. Although the two types of radiation are physically quite different, they are usually considered together, as the site of damage for both is the genetic material (DNA), and the modes of coping with the ensuing radiation damage are fundamentally similar.[24] Although microorganisms are generally resistant to the radiolytic effects of low-level[25] and chronic irradiation, only a few notable species are known to survive high levels of irradiation even remotely similar to those that would be faced on Mars, or in interstellar space. Vegetative cells of *Deinococcus* (formerly *Micrococcus*) *radiodurans* isolates can typically withstand doses of UV light and gamma radiation characteristically withstood by bacterial spores. The dose-response curves show large shoulders that extrapolate to 500 Jm^{-2} for UV radiation and 700 Krad for gamma radiation. Some isolates have been found to survive single doses of 10^4 ergs cm^{-2}, or 10^6 rads.[26-28] In addition, within a core of the Three Mile Island reactor and other commercial reactors, microorganisms have been isolated after exposure to very high levels of radiation. Probably all vegetative cells, even those of the extremely resistant forms, possess similar mechanisms for coping with radiation stress.[29] These mechanisms have been studied intensively in *Escherichia coli* and other well-characterized bacteria. The alterations that occur and that allow organisms to tolerate levels of radiation flux higher by orders of magnitude than those tolerated by *E. coli* are not yet well characterized. In general, both prokaryotes and eukaryotes show greater rates of mutation when subjected to increased UV fluxes, and these mutations are thought to be induced during the processes that repair radiation-induced lesions. Although it seems clear that increased mutagenesis is associated with rapid DNA repair, the repair mechanisms are not well understood for populations subject to UV stress for extended periods of time. It should also be mentioned that the studies of these responses discussed here have focused primarily on vegetative cells; as discussed elsewhere, the situation for the highly resistant bacterial spores might be quite different.

LIFE DETECTION FOR PLANETARY PROTECTION (INCLUDING BIOBURDEN DETERMINATION)

Techniques for assessing the existence of microorganisms have advanced dramatically since pre-Viking days, and these advances will strongly affect bioburden assessment procedures as well as future life-detection experiments. Until the mid 1970s, the major methodology used for detecting microbes was the counting of viable colony-forming units (CFUs) on vari-

ous defined media. NASA procedures carefully outlined the swabbing procedures and media to be used to assess "cleanliness."[30,31] Beginning with epifluorescence microscopy, new methods with greater sensitivity and specificity have rapidly appeared. **The task group strongly recommends that efforts be made to explore current analytical methods for use in bioburden assessment and inventory procedures. New procedures for bioburden assessment must be established before the spacecraft that are designed to detect life are assembled and launched.**

In addition to the epifluorescent microscopic techniques developed for counting viable cells, many other new methods have been developed that involve the detection of specific biomarkers that are components of cells. Such biomarkers may provide a sensitive means for detection without the necessity for release of attached microbes from the substratum (needed for most microscopic counting) or the efficient growth of each propagule. Circumventing the requirement for cultivation is crucial, since it is estimated that fewer than 10 percent of the microorganisms present in most environmental samples have been cultivated. Thus there is a risk that techniques that rely on cultivation will not detect the majority of the microbial population in a given sample.

In addition to obviating the need for cultivation, these techniques are appealing because of their extreme sensitivity. In some cases, single cells can be detected and identified. However, due to this sensitivity, life-detection experiments using these techniques may be compromised if the bioload of the spacecraft is not also monitored using the same technologies.

Viable But Nonculturable Organisms

A recently recognized problem is that some organisms are fully functional even though they are not culturable with the usual microbiological techniques. This has been shown most clearly with the cholera-causing pathogen *Vibrio cholerae*. This organism, when attached to chitin substrata under starvation conditions, is not culturable in any of the standard media, but it is fully infectious if given to a suitable host animal.[32] It is now recognized that nonculturability is a widespread phenomenon in environmental samples. In surface soils, direct microscopic counts of stained bacteria show that less than 1 percent of the organisms seen by epifluorescence microscopy can subsequently be recovered by direct plating and grown to form colonies. Clearly, the previously used procedures for counting viable organisms are insufficient to help assess potential contamination by organisms that could possibly reproduce on another planet or on spacecraft components.

Epifluorescence Microscopy

In the early 1970s epifluorescence microscopy began to be used for the detection of potentially viable (i.e., nucleic acid-containing) microbes. The method involves using fluorescent dyes, such as acridine orange, that bind to DNA and RNA and then directly examining and counting fluorescent particles under UV illumination. Such procedures showed that viable count methodology (e.g., the methods used in bioload assessment for the Viking mission) drastically underestimates the actual microbial population.

Although obtaining acridine dye epifluorescent counts cannot give information as to species composition, coupling fluorescent microscopy to other approaches can do so. Specific oligonucleotide probes labeled with fluorescent dyes can be used to identify and quantitate individual taxonomic groups.[33,34] Such technology may have great importance in the identification and quantitation of targeted groups of microbes during bioburden assessment.

Lipids as Biomarkers

One technique that has recently been widely used to detect and identify microbes is based on the extraction of membrane lipids.[35] Lipids provide two advantages as biomarkers. The extraction of lipids from cells in their environmental matrix is quantitative and allows both a simple purification as well as a concentration step. Extraction has classically been performed with a one-phase chloroform-methanol-water system that requires the use of potentially toxic solvents as well as a prolonged period of exposure to the extraction solutions. Recent use of supercritical fluid carbon dioxide with suitable polar modifiers has made rapid and semi-automatable extraction techniques possible.

Detailed analysis of the extracted lipid biomarkers provides quantitative evidence for the presence of viable components of the microbial community. The polar lipid fraction of the extract is polar by virtue of the presence of primarily phosphate esters. These polar lipid phosphate esters are metabolically labile. During growth or after death, the polar lipids show a relatively rapid turnover by phospholipases inside or external to the cells so that the polar lipid content rapidly disappears from nonliving cells. The dephosphorolated neutral lipid molecular components of the original polar lipids are then readily detected. Consequently, the detection of specific polar lipids provides a quantitative definition of the viable or potentially viable cellular biomass and requires no growth or recovery of intact microbes.

Because different groups of microbes contain identifiable specific patterns of lipid components, detailed examination of the structure of the lipid allows definition of the community structure of the microbial community.

The changes in the lipid component structures also correlate with the nutritional status of the microbiota and with recent exposures to some toxic stresses. Thus the lipid analysis can provide direct evidence of lipid synthetic gene activity as well as the viable biomass, community structure, and nutritional status of the community. Since this technique could provide a means to detect the presence of extant or fossil life on Mars, it is important to prevent potential contamination of spacecraft by specific microbial lipids.

Nucleic Acids as Biomarkers

Nucleic acids are the second group of biomarkers that have received considerable attention in the past several years. Both RNA and DNA provide suitable markers for identifying and quantitating groups of organisms or individual strains of microorganisms. Much of this research in microbial ecology has focused on the use of ribosomal RNAs (rRNAs), both for the identification of microorganisms and for the production of unique nucleic acid probes used for quantitation and for in situ hybridizations. One obvious advantage to using nucleic acids for microbial identification is that limiting amounts of nucleic acids can readily be amplified up to a million-fold, permitting the analysis of only a few molecules of nucleic acid. Amplifications are currently performed either with the polymerase chain reaction, in which a thermostable DNA polymerase is used to amplify a template following denaturation of the template DNA, annealing of suitable primers, and extension of the primed template; or with self-sustained sequence replication, an isothermal mode of amplification modeled after viral replication.

Following the acquisition of nucleic acids, the targeted gene sequences (usually from an rRNA-encoding gene) are analyzed and used to determine the identity of microorganisms in the sample. This information can also be used to design nucleic acid probes for use in future studies and for monitoring the relative abundances of microorganisms in a sample. Since this technology is currently available, life-detection experiments that may use these techniques need to be conducted in an environment that is not contaminated by nucleic acids.

Detection of Spore-forming Bacteria

The swab-and-culture technique used to detect spore-forming microbes as sterilization-resistant contaminants can be improved by biomarker recovery, which obviates the requirement that organisms be cultured for detection. Recovery of 2-4 diamino pimelic acid and/or a signature rRNA sequence could provide a quantitative biomarker for gram-positive spore-forming bacteria.

Detection of Chirality as an Indicator of Bioprocesses

It is particularly important to apply stringent bioload-reduction technology to those missions anticipated to involve the detection of past or present life. One of the most sensitive detection methods will involve the determination of a significantly greater than expected chirality in components of polymers such as peptides. This is one of the most characteristic features of life on Earth. Recent advances in the use of chiral derivatizing agents or stationary phases in column chromatography coupled with the detection of specific analytes on cooled germanium disks (which allow matrix-assisted microscopic Fourier transform infrared spectroscopy) provide ultrasensitive methodology that could be adapted to systems for detecting microbial contamination of spacecraft. It should be mentioned, however, that such techniques require significant amounts of material in comparison to molecular amplification methods such as the polymerase chain reaction.

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Assessment of the 1978 Report

REVIEW

The 1978 report of the then Space Science Board's Committee on Planetary Biology and Chemical Evolution established a quarantine policy for exploratory, one-way missions to Mars, Jupiter, Saturn, Uranus, Neptune, and Titan planned for 1974 to 1994.[1] The recommendation of the 1978 report was that precautionary measures be taken to minimize forward contamination of these planets by terrestrial microorganisms so as not to jeopardize future life-detection experiments.

The criteria used for planetary contamination prior to the 1978 report were those established by international agreement through the Committee on Space Research (COSPAR). They stipulated that the probability of contamination (P_c) should be less than 1×10^{-3} for each planet. The P_c was estimated using a formula that also included the probability of growth (P_g) of a terrestrial microorganism on each of the planets. There was some difficulty in arriving at a sensible and useful P_g, necessitating that the 1978 committee be charged with the task of comprehensively evaluating P_g based on available knowledge of the physical and chemical properties of the surface and atmosphere of each planet and conditions that limit life as we know it.

Although the 1978 committee considered the P_g for all the planets being considered for exploration through 1994, the current report is limited to an evaluation of information and past recommendations for Mars. The 1978 report attempted to evaluate the P_g for three separate regions on Mars and included above- and below-surface subpolar areas and the polar caps.

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Although the committee expressed a reluctance in recommending a particular value for P_g, they argued that while the P_g for Mars is exceedingly low, the probability is not zero. Furthermore, the Viking mission, although useful in arriving at a P_g for subpolar sites, did not offer any insight on geochemical characteristics and the possibility of liquid water at the polar caps. The committee recommended a P_g of less than 10^{-10} for the subpolar regions of the planet within 6 centimeters of the surface, less than 10^{-8} for subsurfaces in subpolar regions, and less than 10^{-7} for the polar ice caps. These ranges for P_g values reflect Viking data for subpolar regions, including those results that indicated the presence of strong oxidants, observed organic compounds, water, and the possibility that liquid water could exist seasonally and diurnally at the polar caps. The P_g values were arrived at subjectively and have become a matter for debate.

It is clear that considerable uncertainty has been engendered by the probabilistic approach to planetary protection. This concern has been restated over the years by virtually every group that has analyzed the problem, and indeed by NASA. Many unknowns must be factored into such elements as the probability of growth of a terrestrial organism on the martian surface, for example, so that estimating the potential for biological contamination of Mars is difficult if not impossible. However, the trend is clear: as we have learned more about Mars, our expectations regarding the likelihood of terrestrial microbial contamination have been reduced, and estimates of the probability of growth have been steadily lowered as a result.

Following the 1978 report, whose recommendations were generally accepted, NASA began to look for ways to simplify planetary protection procedures as they applied to particular upcoming planetary missions, and also to minimize the use of mathematical models and quantitative analyses. These studies culminated in a report to COSPAR in 1984 that greatly deemphasized the probabilistic approach and introduced the concept of categories based on target planet and mission type.[2] This approach directly reflects the degree of concern for a given planet in the context of a particular type of mission.

Five categories of target planet and mission-type combinations and their particular suggested ranges of requirements were proposed in the 1984 report, and these were accepted by COSPAR. The five categories are summarized below; details are contained in the 1984 report (see also Table E.1, Appendix E).

• **Category I** missions include any mission to a target planet that is not of direct interest for understanding the process of chemical evolution. In effect, no protection of such planets (e.g., Mercury, Pluto) is warranted, and no planetary protection requirements are imposed.

• **Category II** missions are all types of missions to those target planets

that are of significant interest for understanding the process of chemical evolution, but for which there is only a remote chance that contamination carried by a spacecraft could jeopardize future exploration. The concern is primarily over unintentional impact, since these missions are not designed to land.

• **Category III** missions are certain types of missions (flyby and orbiter) to a target planet of interest for understanding the chemical evolution and/or the origins of life, or for which scientific opinion suggests a significant chance of contamination that could jeopardize a future biological experiment.

• **Category IV** missions are certain types of missions (mostly probe and lander) to a target planet of interest for understanding chemical evolution and/or the origins of life, or for which scientific opinion suggests a significant chance of contamination that could jeopardize future biological experiments.

• **Category V** missions include all Earth-return missions. The concern is for the protection of the terrestrial system as well as the scientific integrity of the returned sample.

These recommendations, made by NASA, were approved by Subcommission F (life sciences) and subsequently by the executive committee of COSPAR, and they have been implemented by NASA. The task group believes that approval and implementation of these recommended categories constitute a significant step forward in the process of simplifying and implementing planetary protection procedures.

A goal in this report is to reassess current planetary protection guidelines in light of new knowledge and new technology. The task group was asked to comment only on Mars lander missions that do not involve in situ extant life-detection experiments and has tried to do so, although it was admittedly difficult for task group members to exclude life-detection and sample return missions from their thinking. This group's approach, which is somewhat different from that taken in earlier studies, is intended to contribute to planetary studies as they relate to questions about the origins of life, while keeping secure our profoundly important scientific objectives.

RECOMMENDATIONS OF THE TASK GROUP

Forward Contamination

The task group views the problem of forward contamination as separable into two principal issues. The first centers on the potential for growth, in the martian environment, of whatever fractions of spacecraft populations of microorganisms are able to survive transit from Earth to the surface of

Mars. The second involves importation of terrestrial organic contaminants, living or dead, in amounts sufficient to compromise the search for evidence of past or present life on Mars itself.

The guidelines on probability of growth (P_g) issued by the Space Science Board in 1978 were recently reassessed in a 1991 NASA report, *Planetary Protection Issues for the MESUR Mission: Probability of Growth (P_g)*.[3] Comments and estimates made by the contributors point to P_g values for terrestrial organisms on Mars that are probably lower than the 1978 estimates. Their consensus was that an exceedingly small P_g was necessitated by the low probability of liquid water existing on Mars and the low probability of an appropriate terrestrial organism occupying a particular martian environment and growing there. However, P_g was not judged to be zero because of the possibility that suitable martian microhabitats could conceivably exist.

Based on the findings of the MESUR mission workshop on the probability of growth as well as on the arguments presented below, the task group agreed that the P_g value for terrestrial organisms on Mars is so small as to be of no consequence. Therefore, the need for severe reduction of spacecraft bioload solely to prevent the spread of replicating terrestrial organisms on Mars is no longer paramount. However, this is clearly *not* the case as far as contamination of a possible past or extant martian biosphere is concerned. The reduction of bioload on all lander missions to Mars must continue to be seriously addressed. The sophistication of current molecular analytical techniques is such that *single* cells are detectable, and so the issue of spacecraft cleanliness is particularly crucial when life-detection experiments are included in the scientific payload. Aside from considerations related to life-detection experiments, spacecraft cleanliness (particularly the biological-organic burden) is extremely important (1) in order to greatly minimize the introduction of foreign material into any site likely to be of biological interest in subsequent missions, and (2) to minimize contamination of experimental devices that are particularly sensitive to biological and chemical contamination (i.e., optic and spectrophotometric devices).

The deliberations of the task group on the issue of forward contamination hazards posed by the planned set of U.S. and Soviet lander missions summarized in Chapter 2 were greatly aided by NASA's 1991 report on the MESUR mission and by comprehensive briefings given by experts on matters relevant to this issue (see workshop presentations listed in Appendix C). These deliberations led the task group to unanimous concurrence with the following conclusion:

> Forward contamination, solely defined as contamination of the martian environment by growth of terrestrial organisms that have potential for growth on Mars, is not a significant hazard. However, forward contamination

more broadly defined to include contamination by terrestrial organic matter associated with intact cells or cell components is a significant threat to interpretation of results of in situ experiments specifically designed to search for evidence of extant or fossil martian microorganisms.[4]

Based on this consensus, the task group makes the following recommendations for control of forward contamination, each tied to specific mission objectives:

1. Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined; Viking technology may be adequate, but requirements will undoubtedly be driven by the nature and sensitivity of the particular experiments. The rationale for this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter that is deposited at the site by microorganisms or organic residues carried on the spacecraft. This approach, when coupled with molecular analytical methods for assessment of bioload, should allow both elimination of the most troublesome contaminants and an inventory of those few that remain.

2. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures—such as clean-room assembly and cleaning of all components—for reduction of bioload, but such spacecraft need not be sterilized. This recommendation has important implications for the planetary protection program in general, in that it implies that there need be no requirement with regard to orbiter lifetimes if the orbiter is subject to a Viking-level reduction of bioload by clean-room assembly and cleaning.

As discussed above, the task group concurs with the conclusion, expressed in NASA's 1991 report,[5] that the probability of growth of a terrestrial organism on present-day Mars is essentially zero. However, the task group recommends bioload reduction for anything sent to the martian surface. Major advances in our ability to detect cellular material have occurred over the last decade, and future advances will undoubtedly follow. Reducing contamination of the planet by reducing the bioload on landed vehicles will minimize the chances of jeopardizing future experiments designed to detect material of possible biological origin.

These conclusions and recommendations on the issue of forward contamination are based on several considerations discussed earlier in this report. The task group concurs with the MESUR workshop panelists' consensus that P_g is extremely low, and probably significantly below the upper limits estimated by the 1978 committee. Given the likelihood that P_g is

extremely low, the task group sees no utility in further attempts to estimate its probable value in various martian environmental regimes. In the absence of crucial data relating to the potential of terrestrial organisms to survive and grow on Mars, such exercises are purely subjective. Although some progress toward quantification of P_g could perhaps be realized in well-designed laboratory simulation experiments, the task group is not optimistic that the central question of the presence and duration of a liquid water phase in the near-surface martian regolith environment can be unambiguously addressed without more information obtainable possibly only from in situ measurements on Mars itself, or from returned samples—or conceivably from neither.

The task group believes that the recommendations set out above strike an appropriate balance between the obligation for conservatism on the issue of forward contamination insofar as P_g is concerned, and the need to gather the data that will eventually allow that issue to be settled definitively. It is implicit in these recommendations that the approach used in previous attempts to calculate the probability of contamination (P_c) be abandoned. In support of abandoning the method, the task group worked through some sample calculations of P_c to demonstrate the nonutility of the probabilistic approach. P_c is correctly expressed, *per unit of microbial burden*, as the product of P_g and P_t, where P_t is the probability of an organism's survival during transit from Earth surface to Mars surface. P_t is usually expressed as $P_t = P(VT) \times P(UV) \times P(R) \times P(A) \times P(SA)$, with P(VT) and P(UV) representing the probabilities of an organism's surviving exposure to space vacuum and temperature and to ultraviolet radiation, respectively; P(R) the probability of an organism's release from a lander to the martian surface; and P(A) and P(SA) the probabilities of an organism's arriving at the planet and surviving atmospheric entry. Presumption of a successful mission sets P(A) equal to 1 and P(SA) equal to near 1. Data on P(VT) and P(UV) are lacking for most of the recently discovered highly specialized organisms described above, but it is still possible to conservatively estimate their product as 10^{-1} to 10^{-2} or less. (The task group notes that appropriate laboratory simulation experiments to evaluate these probabilities for candidate microorganisms are entirely feasible, since both the spacecraft geometry and the characteristics of its space environment can be well determined.) P(R) is interpreted as the probability of release of that fraction of the total bioburden located on surfaces in direct contact with the martian regolith. With special attention to cleaning such surfaces, perhaps combined with prelaunch UV irradiation, it seems feasible to reduce P(R) to 10^{-2} to 10^{-3} *without* total spacecraft sterilization. Then, even with the 1978 SSB value for P_g of less than 10^{-10}, the product of $P_g \times P_t$ seems unlikely to exceed about 10^{-14} per unit of microbial burden. This nominally allows a large bioload approaching 10^{11} (say, 10^5 organisms per square

centimeter on a spacecraft surface area of 100 square meters) while still retaining the COSPAR value for P_c of 10^{-3}. The task group also notes that this bioload is the *total* microbial burden. Consideration of only those species with capabilities for surviving in the most extreme environments would reduce P_c for them, probably by a relatively large factor. Another factor to consider is the possibility of such extreme environments existing on Mars, some of which may be hospitable to certain organisms. Clearly, if such niches exist, the P_g may be greater for a population of contaminating organisms if they are widely dispersed, thus raising the probability of their encountering a less hostile environment.

It was the intent of the task group to illustrate the uncertainties involved in the probabilistic approach by performing the above calculations. With so many uncertain probabilities multiplied by each other, the likelihood of achieving a meaningful P_c is very low indeed. When these problems are combined with the fact that the range of environments on Mars is not yet known, the futility of assigning a meaningful P_c is further exemplified.

The task group emphasizes that the philosophical intent of the 1978 committee to protect Mars from terrestrial contamination so as not to jeopardize future life-detection experiments on Mars is still profoundly important. Recommendation 1 above deals with the issue of contamination by nonviable but intact cells and biochemical components from terrestrial organisms, independent of whatever low P_g value they may have.

Back Contamination

A detailed assessment of the complex issue of sample return does not lie within the present charge of this task group. Chapter 6 discusses some of the martian environmental unknowns, and the data required to address them, that will be central to evaluation of possible hazards posed by back contamination.

SCIENTIFIC ISSUES—SUMMARY STATEMENT

As previously stated, it is the unanimous opinion of the task group that terrestrial organisms have almost no chance of multiplying on the surface of Mars and in fact have little chance of surviving for long periods of time, especially if they are exposed to wind and to UV radiation. However, current techniques to detect life, such as those that use specific biomarkers, are much more sensitive than techniques used at the time of the Viking mission, making contamination a serious threat to experiments designed to look for life on Mars. With regard to this latter point, the recommendation that landers be sterilized if they carry life-detection experiments, but only have reduced bioloads in other instances, has long-range strategic implica-

tions. Even if there is no organismal growth, local contamination is to be expected around a nonsterilized spacecraft. Clearly a lander should not return to do life-detection experiments at a site where unsterilized spacecraft have landed previously. For these reasons, the task group believes that it is better to err on the side of caution. Thus the task group recommends **that spacecraft be cleaned rigorously to levels that are at least equal if not superior to Viking levels.** It does not believe that such constraints are unduly restrictive to subsequent Mars exploration.

The task group also recommends that modern methods of bioburden assessment and tabulation be developed for spacecraft destined for Mars missions.

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1. Space Science Board, National Research Council. 1978. *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan.* Committee on Planetary Biology and Chemical Evolution. National Academy of Sciences, Washington, D.C.
2. DeVincenzi, D.L., and P.D. Stabekis. 1984. "Revised Planetary Protection Policy for Solar System Exploration." *Adv. Space Res.* 4:291-295.
3. Klein, H.P. 1991. *Planetary Protection Issues for the MESUR Mission: Probability of Growth (P_g).* NASA conference publication. NASA Ames Research Center, Moffett Field, Calif.
4. See Klein, H.P., 1991.
5. See Klein, H.P., 1991.

6

Additional Recommendations

RECOMMENDATIONS FOR RESEARCH

Any future changes in recommendations made to ensure planetary protection, especially for piloted or sample return missions, will depend on the acquisition of new data. To this end, the task group believes that a sequence of unpiloted missions to Mars, undertaken well before a piloted mission, is imperative. One of the keys to deciphering the question of life on Mars lies in knowing where to look; the Viking landing sites were not optimum in this sense. They were selected primarily on the basis of considerations of spacecraft safety, rather than scientific potential. Because of this, we have a paucity of critical data needed to assess the possibility of contemporary or ancient life on Mars. **Data should be gathered from a broad spectrum of sample sites with measurements focusing on data most likely to contribute to a better understanding of the probability of life on Mars**. Among the classes of information needed are chemical (e.g., data on mineralogy, soil pH), physical (e.g., data on temperature, light—qualitative and quantitative), and hydrological (i.e., data on the status of water availability, historical and current). Until such data are available, it will be impossible to make informed decisions concerning landing sites for in-depth biological study. Such data also will greatly affect the ability to make future decisions concerning the standards of rigor required for spacecraft cleanliness and possible sterilization.

The term *planetary protection* encompasses two very distinct concepts: the forward contamination of Mars and the back contamination of Earth. In

this report, the task group specifies the planetary protection policy it believes appropriate with regard to forward contamination, i.e., (1) sterilization in missions with life-detection goals and (2) a general rigorous reduction of bioload in all others. Although these differ from the 1978 recommendations, the rationale is grounded in the scientific consideration of risk assessment (i.e., that the survival and/or growth of terrestrial organisms transported to Mars is highly unlikely) and aspects that threaten mission goals (i.e., that life-detection experiments may be compromised by spacecraft contaminants). However, the task group believes that there are areas in which the lack of current available data limits both the formulation of recommendations for planetary protection and the potential for mission success.

To correct for this, the collection of certain data sets and the adoption of the overall approach outlined below are strongly recommended. These recommendations emphasize the need to firmly characterize the existing environmental conditions and the geochemical composition of Mars. This information will serve two purposes: (1) it will allow informed estimates of the potential for life (as we currently understand it) to exist on Mars and of the potential threat of contamination posed by backward transport of such life to Earth, and (2) it will identify those locations where life-detection missions should be sent. It is essential that these studies precede any life-detection or piloted missions to the martian surface as well as any missions designed to return samples to Earth.

Collection of Essential Data

Viking provided us with pictures of a martian surface varying widely in its geomorphological features. Unfortunately, the Viking landers were located in relatively featureless, exposed areas of the planet chosen on the basis of landing safety. Therefore the data collected by these landers reflect only this harsh physical and chemical environment. To establish a policy to ensure planetary protection from back contamination, we need data from locations with a much greater potential to support life. Measurements taken from a variety of sites might allow specification of which martian environments might be least hostile to life; these will be very important sites for collection of relevant data regarding environmental variables (e.g., water, temperature, radiation) that might be used to predict the existence or survival of life forms. This approach would minimize any argument that the potential for life (and therefore for the back contamination of Earth) is underestimated by models incorporating data on only the harshest or least hospitable conditions. These same issues are significant in the placement of

life-detection landers on the planet; sites with the greatest potential to support life now or in the past must be identified.

It was not the charge of this task group to identify locations or specific measurements or experiments for future missions; that is left to others. However, the recommendation to locate martian landers in sites with the maximal likelihood of fostering life might be further refined to suggest that these sites may be determined from our rudimentary understanding of Mars and our growing, but extensive, knowledge of the basic requirements of life. The existence of contemporary life on Mars has been presumed unlikely based on the lack of water, low temperatures, high UV flux, strongly oxidizing surface chemistry, and other parameters. If these factors are assumed to limit life, landers should be located in those areas where it is suspected that these conditions are least severe now or were so in the martian past. Given the consideration of water, a suitable site might lie in the polar regions, in one of the fluvial features associated with earlier hydrological activity, and/or in an area where geothermal vents are most likely to be found.

In addition to selecting sites appropriate on a large scale, it is important to consider the subsurface of Mars. Temperature, UV attenuation, and other factors vary with depth and season and may offer a stable or transient refuge for life. Thus within a site it may prove to be important to design data collections that probe below the readily accessible surface, thus providing information on subsurface environments.

The surface of Mars may well be highly heterogeneous, even more so than is now suspected. Microenvironments—whether on the surface or in isolated vents, cracks, or layers of the subsurface—may exist now or may have existed in the past. Properly designed experiments may be able to address the issue of spatial and (perhaps) temporal heterogeneity and its possible relationship to our ability to evaluate the biotic and abiotic status of a given site.

Future sample return missions, piloted missions, and their associated quarantines will benefit from a planetary protection policy predicated on an approach that yields the least conservative estimates of existing martian life. Collection of the appropriate data should allow the scientific community to amend recommendations for a planetary protection policy for back contamination, perhaps resulting in recommendations similar to those that this task group has made for altering current policy on forward contamination. In addition, the determination of current or inferred past geophysical conditions on Mars may help in identifying locations where life-detection missions should be sent. This information would certainly increase the likelihood of success in meeting the goals of those missions.

Assessment of Spacecraft Bioload

The task group's recommendation to "reduce" bioload on spacecraft in all missions and to sterilize those spacecraft used in life-detection missions assumes the use of Viking procedures.

However, the task group recommends against the use of the Viking protocols for assessment of spacecraft bioloads after these cleaning procedures have been done. The 1980 guidelines for Viking bioload assessment[1] are outdated and far less sensitive than the methods that will most likely be used to detect martian life. We now know that many organisms are undetected by standard culturing methods and that bioload estimates may, in fact, represent only 1 percent of the organisms actually present.

The task group recommends that efforts be initiated immediately to adopt state-of-the-art methods for use in the determination of bioload. These methods should be the same as those most likely to be used in actual life-detection experiments conducted on Mars. They would, therefore, have the advantage of being sensitive enough to recognize low levels of biomarkers and of obviating the need to culture microorganisms. Since a major concern driving the task group recommendations is preventing the invalidation of life-detection missions by spacecraft-borne contaminants, it is critical that methods for assessing bioload be compatible with methods for detecting life: methods for both assessment and detection must reflect the same limits and sensitivity. Although it is not reasonable to demand that these methods be used for upcoming launches, it is imperative that they be used for missions involving life detection and that a program to implement them be established as soon as possible.

Data on bioloads of Viking components and spacecraft are not relevant to current life-detection procedures. **It is absolutely necessary that NASA investigate the bioload of component parts with state-of-the-art methods.** Early funding of research designed to address the issue of detecting biomarkers after application of various cleaning procedures could lead to the use of less stringent means of reducing bioload. It would also allow NASA to customize procedures for specific life-detection methods. As there currently is no budget for this type of activity, the task group recommends that NASA's Office of Planetary Protection be given funds for the purpose of bioload research.

RECOMMENDATIONS CONCERNING OTHER ISSUES

Piloted Versus Unpiloted Missions

Plans for future missions to Mars include bringing samples back to Earth as well as landing humans on Mars. Although humans may be effective, and perhaps even necessary, for the detection of past life (e.g., by the

collection and analysis of fossil-containing sediments and rocks), missions carrying humans will contaminate the planet, thereby making the search for extant life much more difficult. It is therefore critical that a major effort be made to determine whether there are places in local martian environments, such as active hydrothermal areas, where life might plausibly survive, and to more closely examine these areas robotically, before contamination by humans occurs. Relevant evidence could be obtained either by bringing back samples to Earth for examination or by making in situ measurements. Realistically, it is not likely that there will be near-term opportunities to bring samples back to Earth. If sample return is not possible, then every effort should be made to obtain chemical and physical measurements germane to the issue of life on Mars.

Societal and Legal Issues

The issues of forward and back contamination involved in missions to Mars have societal and legal implications at international levels. They are serious enough concerns in today's society to warrant discussion here.

A dominant force in the 1980s was the powerful wave of public concern about environmental problems. The task group believes that these concerns are real and continuing and should be given serious attention by NASA. A substantial number of national and international organizations, active and well funded, are on the alert for environmental abuse. There is every reason to take seriously the concern (already expressed in some cases) about contamination of Mars and almost certainly about the issue of back contamination of Earth by martian samples. Although public concern over such issues is often sincere and useful, it at times becomes distorted and exaggerated in the media, sometimes in a sensationalist and nonproductive way, leading to public misunderstanding and opposition.[2] In some cases, these concerns have led to lengthy court actions. To forestall such unnecessary confrontation, **the task group recommends that NASA make every attempt to inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return**. The task group thinks that there is not likely to be great public concern over the question of outbound contamination, especially if the public understands the scientific objectives and is aware that the issue of contamination has been addressed (and that appropriate precautions are being taken). The better the effort at public education and the earlier it begins, the smaller the likelihood that there will be public concern and negative reaction. In the case of sample return missions, the task group believes that the potential for negative reaction is much greater and that the need for public education and involvement is therefore even greater.

In addition to the scientific aspects of planetary protection that need to

be considered, there are also legal issues that must be addressed, involving international restrictions as well as federal, state, and local statutes that may come into play. A number of relevant statutes and regulations are written by agencies as diverse as the Department of Agriculture, the U.S. Public Health Service, the Department of Interior, and the Environmental Protection Agency, all of which deal with the exposure of American citizens to hazardous or toxic materials. International groups such as the United Nations, the World Health Organization, and the International Labor Organization have also attempted to address questions involving protection of Earth's environment and minimization of risk to populations from space exploration activity. In most cases, these documents lack specific details and contain almost no scientifically based discussion of risk of contamination, precautions needed, or procedures to follow in case of an accident. There are currently no binding international agreements concerning forward or back contamination.[3] **The task group believes it is essential (1) to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and (2) to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars, as well as provide protection for Earth and her inhabitants. NASA should make a strong effort to obtain international agreement for planetary protection issues.** A strong international component will help assuage possible domestic concern.

NASA should, even at this early date, acknowledge the problems outlined above and reestablish the kind of planetary protection program that existed through the Viking Program. Although a planetary protection officer exists, there is no budgeted program to implement needed planetary protection research, public education programs, and the like. **The task group recommends that NASA correct this situation as soon as possible by redefining the responsibilities and authority of its planetary protection officer and by providing sufficient resources to carry out the recommendations made in this report.**

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2. DeVincenzi, D.L., H.P. Klein, and J.R. Bagby. 1991. *Planetary Protection Issues and Future Mars Missions.* NASA conference publication. NASA Ames Research Center, Moffett Field, Calif.
3. Robinson, George S. 1991. "Exobiology and Planetary Protection—The Evolving Law," an unpublished paper presented to the Space Studies Board Planetary Protection Workshop, NAS Beckman Center, September 13.

Appendixes

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Correspondence Documenting
Start of Task

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NASA

National Aeronautics and
Space Administration

Washington, D.C.
20546

Reply to Attn of   **SBR**

JUL 16 1990

Dr. Louis Lanzerotti
Space Studies Board
National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C.   20418

Dear Dr. Lanzerotti:

NASA's efforts in planetary protection seek to preserve planetary conditions for future
biological and organic constituent exploration, and to protect Earth and its biosphere from
potential extraterrestrial sources of contamination. As stated in the relevant NASA Management
Instructions, the Space Science Board has been the primary group advising NASA on this issue
over the years, and continued advice on planetary protection from the Space Studies Board will
be needed to ensure that NASA policy in this area remains robust.
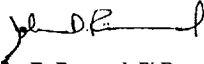
As you know, in the last year NASA has been working with the National Space Council to
define a Space Exploration Initiative that envisions future human missions to the moon and
Mars. In current planning, human missions to Mars are preceded by robotic missions that will
be defined in the early-1990's and may include Mars landers and the launch of a Mars Sample
Return near the turn of the century. In addition, the Soviet Union is currently planning a
mission that will place small landers on Mars in 1994, while the US Mars Observer mission is
still in operation around that planet. NASA planetary protection policy and its application to
future Mars missions will be dependent on the advice of the Space Studies Board, the lessons of
intervening Mars missions, and on NASA's ability to apply this information to follow-on
planning for an intensive program of Mars exploration.

Due to the timing of the planning for additional Mars missions, it would be appropriate to begin
to study the question of Mars planetary protection within the Space Studies Board as soon as
possible. Because of the potential for planetary protection requirements to significantly impact
Mars missions, especially in light of the back-contamination issues attendant to a sample return,
it will be necessary to address Mars planetary protection issues in a dedicated fashion over the
next several years. Such a study is consistent with the recommendations of the recent National
Research Council review of the planning for the Space Exploration Initiative, which specifically
raised planetary protection as an issue. An initial report to NASA on Mars planetary protection
prior to the 1992 COSPAR meeting would also provide us with a robust US position as we
consider the nature of planetary protection on the Soviet Mars '94 mission, prior to its launch.

The nature of the planetary protection question has certainly changed in the years since the
Apollo and Viking missions, but new thoughts about life on Mars and the growing
environmental awareness of the populace will continue to make planetary protection a
complicated issue in the future. Certainly it must be addressed as part of a responsible program
of exploration. I will be happy to work with you to determine the nature of the study approach
to be taken by the Space Studies Board, and the timing for the study process and the reporting
of results.
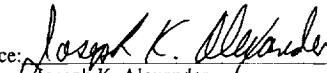
Your help in addressing the question of Mars planetary protection is greatly appreciated. I look forward to helping you to define your study efforts in this area. Please contact me (453-1527) if you need further information about this request.

Sincerely,

John D. Rummel, PhD
Planetary Protection Officer
Office of Space Science and Applications

Concurrence: Joseph K. Alexander

cc:
S/Dr. Fisk
  Mr. Alexander
SB/Dr. Nicogossian
SL/Dr. Briggs
NRC/Mr. Kastel

NATIONAL RESEARCH COUNCIL

COMMISSION ON PHYSICAL SCIENCES, MATHEMATICS, AND APPLICATIONS

2101 Constitution Avenue   Washington, D.C. 20418

SPACE STUDIES BOARD   October 22, 1990

Dr. John D. Rummel
Planetary Protection Officer
Office of Space Science and Applications
NASA
Washington, D.C. 20546

Dear Dr. Rummel:

This is to acknowledge your letter of July 16, 1990 requesting advisory assistance from the Space Studies Board concerning planetary protection issues associated with future Mars missions and the Space Exploration Initiative. I understand that you, Joe Alexander and Wes Huntress have met with members of the SSB staff to discuss this request. The following summarizes, as I understand them, the major points raised in that discussion and describes how the Space Studies Board proposes to respond to your request.

According to NASA Management Instructions, the Space Studies Board is the group charged with providing advice to NASA concerning Planetary Protection Issues. The last time the Board provided a comprehensive set of advice on Planetary Protection was in the 1978 report, *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune and Titan*. The most recent recommendations from the Board concerning Mars were in 1985 in response to a request from NASA to provide advice on the categorization of the Mars Orbiter mission.

It is our understanding that in its present request, NASA would like the Board to focus on three major issues:

(1)   To assess the status of the 1978 report in terms of what recommendations have been implemented and what remains to be done.

(2)   To describe what is known today concerning Mars and planetary protection issues, including back contamination.

(3)   To make recommendations concerning what research should be undertaken to address those questions and issues that are relevant to current planetary protection concerns.

In carrying out the above study, the board will take into account current activities such as the planned USSR 1994 Mars mission and the U.S. plans for both robotic and human missions to Mars in the next century.
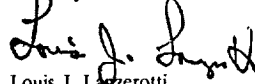
To conduct the above study, we propose the following. Under the direction of the Board, a panel of experts will be selected and appointed. This panel of experts will concentrate on those issues listed above in a workshop to be held in the summer of 1991. In addition to the panel of experts, additional individuals will be invited to participate in the workshop including NASA personnel, representatives from Europe, Japan and the Soviet Union. Given the

Dr. John D. Rummel
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COSPAR involvement in planetary protection, it would also be desirable to include some representatives from that body. It is likely that the workshop will be preceded by one or two organizational meetings of the panel. Following the workshop, we expect that there might be additional meetings to finalize the panel's report. We expect that the report could be published and transmitted to NASA by the Spring of 1992 in time for the 1992 COSPAR meeting.

I understand that at the present time, support for this effort has yet to be resolved. I hope that NASA will find this approach responsive to your needs. I and the Board staff will continue to work with you as we make plans for this effort.

Sincerely,

Louis J. Lanzerotti
Chairman
Space Studies Board

cc: L. Fisk
     J. Alexander
     W. Huntress

NASA

National Aeronautics and
Space Administration

Washington, D.C.
20546

Reply to Attn of: **SBR**   APR 10 1991

Dr. Louis Lanzerotti
Space Studies Board
National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C.   20418

Dear Dr. Lanzerotti:

Thank you for your letter of October 22, 1990, in which you outline your proposal to respond
to the NASA request of July 16, 1990 for advisory assistance on issues concerning Mars
planetary protection.

The overall approach you have outlined for the Board's study of planetary protection issues is
appropriate:
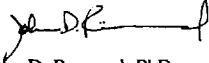
1) Assess the status of the 1978 report and the implementation of its recommendations,

2) Describe the current knowledge of issues related to Mars planetary protection, and

3) Recommend research to be undertaken to address the questions and issues relevant to current
planetary protection concerns.

In pursuing this approach for the long term, it is clear that the process will need to be iterative.
Some of the recommendations of the 1978 report remain to be implemented, but it is also likely
that the perspective gained over the intervening 13 years will lead the Board to reshape some of
those recommendations. Thereafter, results of research conducted to implement the new
recommendations and the return of data from upcoming Mars missions (Mars Observer, and
potentially, Mars '94) will enable an update of the Board's advice in time to guide requirements
for future US lander and sample return missions.

Unfortunately, the same opportunity for an update will not be available to guide US policy with
respect to the USSR Mars '94 mission, our potential participation in that mission, and our
expectations of planetary protection provisions to be taken by the Soviets. Because of the
expected timing of the mission, I believe it is important to receive the Board's advice on this
matter as soon as practicable. Accordingly, as one of the results of the workshop planned for
the summer of 1991, I am requesting that the Board provide an update of planetary protection
recommendations for Mars landers, based on presently available data. These recommendations
will provide an update to the requirements for planetary protection and the measures taken in
conjunction with the Viking landings on Mars, and will assist NASA in the formulation of a
policy applicable to landing missions that may be planned or launched in the near-term.

The assistance of the Board in addressing these questions is greatly appreciated. I look forward to working with you in carrying out this study.

Sincerely,

John D. Rummel, PhD
Planetary Protection Officer
Office of Space Science and Applications

cc:
S/Dr. Fisk
  Mr. Alexander
SB/Dr. Nicogossian
SL/Dr. Huntress
NRC/Dr. Allen
     Ms. Purcell

B

Biographical Sketches of
Task Group Members

KENNETH NEALSON (*Chairman*) is the Shaw Distinguished Professor of Biology at the University of Wisconsin-Milwaukee, an adjunct professor of bacteriology at the University of Wisconsin-Madison, and associate director of the Center for Great Lakes Studies in Milwaukee, a center of excellence of the University of Wisconsin system. Before moving to Wisconsin in 1985, he was a professor of oceanography at the Scripps Institution of Oceanography, where he served for 12 years. During that time, he received a Guggenheim fellowship and spent 1 year on sabbatical leave in the marine chemistry department at the University of Washington in Seattle. His area of research is the biogeochemical cycling of metals in aquatic environments. He has served on two previous Space Studies Board (SSB) committees, chaired by L. Margulis and H.P. Klein. He served for 3 years as a panel member for the Exobiology Program and has been involved for over 10 years with a summer NASA research training program funded by the Biospherics Program at NASA. He has been a distinguished lecturer at 10 different U.S. universities and has been named as a distinguished lecturer of the Australian Microbiology Society for 1992. He received a B.S. in biochemistry and a Ph.D. in microbiology, both from the University of Chicago, and did postdoctoral work in biochemistry at Harvard University.

JOHN BAROSS is an associate professor of oceanography at the University of Washington, Seattle, where he has been since 1985. Baross has been a member of the American Society for Microbiology committees on microbial ecology and numerical taxonomy, a member of the Theoretical, Experimental and Analytical Working Group, RIDGE (Ridge Inter-disci-

plinary Global Experiments), and a Scientific Advisory Board member (alternate) for the Mount St. Helens National Volcanic Monument. Baross is currently a member of the Technical Advisory Panel for the Mount St. Helens National Volcanic Monument. His research interests include the ecology and physiology of hyperthermophiles from marine and terrestrial volcanic environments, the possible relationship between submarine hydrothermal vents and the origins of life, and estuarine microbial ecology. Baross received a B.S. in microbiology and chemistry from California State University at San Francisco in 1964 and a Ph.D. in marine microbiology from the University of Washington in 1973.

MICHAEL CARR is a geologist with the U.S. Geological Survey in Menlo Park, California, where he has been since 1962. During the last 20 years, Carr has focused on studying Mars, particularly its volcanic and climatic history. He was a member of the Mariner-9 imaging team and leader of the Viking Orbiter imaging team. He is currently a member of the Galileo imaging team and is an interdisciplinary scientist on Mars Observer and a co-investigator on the Russian Mars-94 mission. He chairs the Mars Science Working Group that advises NASA on the strategy for future robotic exploration of Mars. Carr received a B.Sc. in geology from the University of London in 1956 and a Ph.D. from Yale University in 1960.

ROBERT PEPIN is a professor of physics at the University of Minnesota, where he has been since 1965, except for a 3-year absence from 1974 to 1977 as director of the Lunar and Planetary Institute (then the Lunar Science Institute) in Houston, Texas. Pepin was a member of the SSB's Committee on Planetary and Lunar Exploration (COMPLEX) from 1983 to 1988, served as its chairman and as a member of the SSB from 1985 to 1988, and was a 1987-1988 member of the SSB ad hoc Committee on Cooperative Exploration of Mars. He has been a member of several committees and boards that advise NASA and the Universities Space Research Association, was mission control science advisor for lunar surface operations during Apollo missions 14-17 in 1970-1972, and currently sits on the Jet Propulsion Laboratory's Galileo Review Board. He is currently a principal investigator in NASA's Planetary Materials and Geochemistry Research Program. He was awarded the NASA Medal for Exceptional Scientific Achievement in 1971. Pepin received a B.A. in physics from Harvard University in 1956 and a Ph.D. in physics from the University of California at Berkeley in 1964.

THOMAS SCHMIDT is an assistant professor of microbiology at Miami University, a position he accepted in 1991 after serving as a postdoctoral fellow at Scripps Institution of Oceanography, the Center for Great Lakes Studies, and Indiana University. Schmidt's research focuses on the development and application of molecular biological approaches to the study of naturally occurring microbial populations, including unculturable microor-

ganisms. He has served as an outside reviewer for NASA's Exobiology Program and the Department of Energy's Subsurface Science Program. Schmidt received a B.S. in biology from the University of Michigan and an M.S. and a Ph.D. (1985) in environmental biology and microbiology from Ohio State University.

JODI SHANN is an assistant professor of biology at the University of Cincinnati, where she has been since 1988. She currently serves on environmental advisory and education committees at the local, national, and international level. She received a B.S. in marine science from Stockton State College in 1976, an M.S. in plant and soil science from the University of Rhode Island in 1981, and a Ph.D. in Botany from North Carolina State University in 1986. From 1986 to 1988, Shann held a Department of Energy postdoctoral fellowship at the University of Georgia and the Savannah River Site.

J. ROBIE VESTAL is a professor of biological sciences and a professor of environmental health at the University of Cincinnati. His research interests include how microbial communities function in nature. He has studied microbial communities in Arctic lakes and in soils contaminated with hazardous waste, cryptoendolithic (hidden within rock) communities in Antarctica, mangrove-degrading communities in the Bahamas, and most recently, decomposer communities in municipal solid waste compost. He has also investigated microbial survival under simulated martian conditions. He earned a Ph.D. in microbiology at North Carolina State University and an M.S. at Miami University. His postdoctoral research at Syracuse University involved the biochemistry of *Thiobacillus ferrooxidans*. Vestal has served on many local and national committees and was recently chair of the Divisional Advisory Committee of the National Science Foundation's Division of Polar Programs.

DAVID C. WHITE has been a University of Tennessee/Oak Ridge National Laboratory distinguished scientist and professor of microbiology and ecology since 1986. He is currently director of the Center for Environmental Biotechnology, where his research into assessment of the microbial ecology of biofilms and subsurface sediments has been used in monitoring microbial biofilms in contained life support systems and in protection of potable water systems for space habitability. He has served on NASA and SSB committees for planetary biology and chemical evolution, global habitability, and major directions in space science and has won awards from the Environmental Protection Agency and Department of Energy for scientific achievement in work on subsurface science and bioremediation. He received an A.B. from Dartmouth College in 1951, an M.D. from Tufts Medical School in 1955, and a Ph.D. in biochemistry from Rockefeller University in 1962.

RICHARD S. YOUNG is a consultant who has worked for more than 30

years in the area of space life sciences (primarily exobiology and planetary protection and controlled ecological life support systems). He is a former manager of NASA's Exobiology Program and was NASA's planetary protection officer in the 1960s and 1970s. He is currently chairman of the Exobiology Discipline Working Group and is a member of several committees in the areas of exobiology, controlled ecological life support systems, and planetary protection. He has been very active in the Committee on Space Research (COSPAR), Life Sciences for many years, and he holds a research professorship at the Florida Institute of Technology. He has an A.B. degree in biology from Gettysburg College, an honorary D.Sc. from Gettysburg College, and a Ph.D. from Florida State University.

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Space Studies Board
Planetary Protection Workshop

WORKSHOP AGENDA

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FINAL AGENDA
SPACE STUDIES BOARD PLANETARY PROTECTION WORKSHOP
NATIONAL ACADEMY OF SCIENCES
ARNOLD AND MABEL BECKMAN CENTER
LECTURE ROOM
IRVINE, CALIFORNIA

SEPTEMBER 9-13, 1991

WORKSHOP OBJECTIVES:

(1) To establish a historical perspective for Planetary Protection requirements

(2) To review existing Guidelines and SSB Recommendations

(3) To examine new scientific evidence pertinent to Mars Exploration and Planetary Protection Requirements

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Day 1 - Monday, September 9, 1991

7:30 - 8:30 a.m.	**BREAKFAST - BECKMAN CENTER REFECTORY**	

8:00 - 9:00	**REGISTRATION**	

9:00	Welcome - Introductions - Workshop Objectives	Ken Nealson Chairman
9:30	Review and Discussion of NASA Request	John Rummel NASA Hdqtrs
10:00	Historical Perspective and Some Issues Pertinent to Planetary Protection Policy	H. P. (Chuck) Klein Santa Clara Univ
10:30	Review and Discussion — Current Planetary Protection Categories and Guidelines	John Rummel NASA Hdqtrs

11:00	NASA Mars Exploration Planning - Code SL	Joe Boyce NASA Hdqtrs
11:45	NASA SEI Mars Exploration Planning - Code RZ	Lewis Peach NASA Hdqtrs
12:15 p.m.	**LUNCH - BECKMAN CENTER REFECTORY**	
1:15	European Science Community Perspectives on Planetary Protection Issues	Claudia Lindberg Inst Aerospace Medicine FRG
1:45	USSR Mars Planning - Mars-94 and Beyond	Michail Ivanov USSR Academy of Sciences
2:30	**B R E A K**	
2:45	Overview of ARC 1990 Planetary Protection Workshop - Purpose, Major Findings, Outstanding Issues	Don DeVincenzi ARC
3:15	Overview of P_s Workshop - Purpose, Major Issues, Conclusions	HP (Chuck) Klein
3:45	Summary of Scientific Issues and Discussions P_s Workshop	Mary Lynn Perille-Collins U Wisconsin Milwaukee
4:30	Antarctic Research Findings Pertinent to Mars/Planetary Protection Issues	Diana Freckman U California Riverside
5:15	**ADJOURN**	

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Day 2 - Tuesday, September 10, 1991

8:00 - 9:00 a.m.	**BREAKFAST - BECKMAN CENTER REFECTORY**

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8:00 - 8:45 Optional Viewing — "Life in Ice"
Room II-D

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9:00	**"MARS THE PLANET"**	Michael Carr USGS Bob Pepin Univ Minnesota
	Mars Science Working Group Activities Concerning Forward and Back Contamination	Michael Carr
	Surface Temperatures	Michael Carr
	Radiation Types and Flux at the Surface	Jim Gooding JSC
	Soil Chemistry	Ben Clark Martin Marietta
	Mars Meteorites	Jay Melosh Lunar & Planetary Lab

12:00 p.m.	**LUNCH - BECKMAN CENTER REFECTORY**

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12:15 - 1:00 Optional Viewing — "Life in Ice"
Room II-D

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1:00	"LIFE IN EXTREME ENVIRONMENTS"	Ken Nealson
	Low Water Stress	Larry Hochstein ARC
	Stability Relations of Water	Bruce Jakosky Univ of Colorado
	Low Temperature Stress	Art Yayanos Scripps
	Low Pressure (Vacuum) Stress	TBD
	UV and Radiation Stress	Richard Setlow Brookhaven Ntl Lab
	Sporulation and Resting Forms and the Survival Capacity	Philipp Gerhardt Michigan State
	Detection of Nucleic Acids	Tom Schmidt Miami Univ

5:30	ADJOURN

6:30	COCKTAILS AND DINNER - BECKMAN CENTER PATIO

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Day 3, Wednesday, September 11, 1991

8:00 - 9:00 a.m.	BREAKFAST - BECKMAN CENTER REFECTORY

9:00 a.m.	Current Scientific and Technological Issues Associated With Sample Return Missions	Roger Bourke JPL
9:45	Alternative Sterilization Methods	Bob Howell Bionetics Corp

10:30	Societal Issues Associated with Planetary Protection/Back Contamination	Margaret Race UC Berkeley
11:15	Legal/Regulatory Issues Associated with Planetary Protection	George Robinson Smithsonian
12:00	Discussion and Wrap-Up	Ken Nealson Chairman

12:30	**LUNCH - BECKMAN CENTER REFECTORY**

EXECUTIVE SESSION

1:15 p.m.	Discussion of Workshop Presentations, Review of Report Outline, Writing Assignments
	Writing Session
5:00	**ADJOURN**

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Day 4, 1991, Thursday, September 12, 1991

8:00 - 9:00 a.m.	**BREAKFAST - BECKMAN CENTER REFECTORY**

9:00	Plenary Session - Discussion of Writing Assignments and Identification of Issues and Problems
9:30	Writing Sessions

12:00 p.m.	**LUNCH - BECKMAN CENTER REFECTORY**

4:00	Plenary Session - Review Progress, NASA Request, and Task Group Charge

5:00   **ADJOURN**

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Day 5. Friday. September 13. 1991

8:00 - 9:00 a.m.   **BREAKFAST - BECKMAN CENTER REFECTORY**

9:00   Writing Sessions

12:00 p.m.   **LUNCH - BECKMAN CENTER REFECTORY**

1:00   Plenary Session - Review of Report, Identification of Outstanding Issues
                       Additional Assignments, Determine Schedule for Completion of Report,
                       Identify Future Meeting Dates

3:00   **ADJOURN**

WORKSHOP PARTICIPANTS

Task Group Members

Kenneth H. Nealson, Chairman, Center for Great Lakes Studies, University of Wisconsin
John Baross, School of Oceanography, University of Washington
Michael Carr, Branch of Astrogeology, U.S. Geological Survey
Robert Pepin, School of Physics and Astronomy, University of Minnesota
Thomas Schmidt, Department of Microbiology, Miami University
Jodi Shann, Department of Biological Sciences, University of Cincinnati
David White, Environmental Sciences Division, Oak Ridge National Laboratory
Richard Young, Private Consultant, Kennedy Space Center

Joyce M. Purcell, Executive Secretary, Space Studies Board

Space Studies Board Liaison Members

James P. Ferris, Department of Chemistry, Rensselaer Polytechnic Institute
Louis J. Lanzerotti, AT&T Bell Laboratories

NASA Participants

Joseph M. Boyce, Office of Space Science and Applications, NASA Headquarters
Don DeVincenzi, Space Science Division, NASA Ames Research Center
Michael Duke, Lunar and Mars Exploration Office, Johnson Space Center
Jim Gooding, Solar Systems Exploration Division, Johnson Space Center
Larry Hochstein, Planetary Biology Branch, NASA Ames Research Center
Lewis L. Peach, Jr., Office of Aeronautics, Exploration, and Technology, NASA Headquarters
John D. Rummel, Office of Space Science and Applications, Life Sciences Division, NASA Headquarters
Perry Stabekis, Programs and Flight Missions Department, NASA Headquarters

Other Invited Participants

Roger Bourke, Jet Propulsion Laboratory, California Institute of Technology
Benton C. Clark, Civil Space and Communications, Martin Marietta Corporation

Diana Freckman, Department of Nematology, University of California
Philipp Gerhardt, Department of Microbiology and Public Health, Michigan
    State University
Robert Howell, Bionetics Corporation, NASA Ames Research Center
Bruce Jakosky, Laboratory for Atmospheric and Space Physics, University
    of Colorado
Harold P. Klein, Biology Division, Santa Clara University
Jay Melosh, Lunar and Planetary Laboratory, University of Arizona
Mary Lynn Perille-Collins, Center for Great Lake Studies, University of
    Wisconsin at Milwaukee
Margaret Race, Botanical Gardens, University of California, Berkeley
George S. Robinson, Office of the General Council, Smithsonian Institution
Richard B. Setlow, Biology Department, Brookhaven National Laboratory
A. Aristides Yayanos, Scripps Institution of Oceanography, University of
    California, San Diego

Foreign Participants

Michail B. Ivanov, Institute of Microbiology, USSR Academy of Sciences
Claudia Lindberg, Biophysics Division, Institute for Aerospace Medicine,
    Köln, Germany

WORKSHOP PRESENTATIONS AND RESOURCE MATERIALS

Presentations

NASA Planetary Protection Policy and Future Mars Mission Requirements—
    Review of the NASA Request, presentation by John D. Rummel, Plan-
    etary Protection Officer, NASA Headquarters, September 9, 1991
Historical Perspective and Some Issues Pertinent to Planetary Protection
    Policy, presentation by Harold P. Klein, Santa Clara University, Septem-
    ber 9, 1991
NASA Planetary Protection Policy and Implementation, John D. Rummel,
    Planetary Protection Officer, NASA Headquarters, September 9, 1991
NASA Mars Exploration Planning (Code SL), Joseph M. Boyce, NASA
    Headquarters, September 9, 1991
NASA SEI Mars Exploration Planning (Code RZ), Lewis L. Peach, Office
    of Exploration, September 9, 1991
European Science Community Perspectives on Planetary Protection, presen-
    tation by Claudia Lindberg, Institute for Aerospace Medicine, DLR, Köln,
    Germany, September 9, 1991
USSR Mars Planning, presentation by Michail Ivanov, USSR Academy of
    Sciences, September 9, 1991

A Summary of the NASA Workshop on Planetary Protection Issues and Future Mars Missions, Donald L. DeVincenzi, NASA Ames Research Center, September 9, 1991

Overview of Probability of Growth (P_g) Workshop—Purpose, Major Issues, Conclusions, presentation by Harold P. Klein, Santa Clara University, September 9, 1991

Mars Surface Temperatures, presentation by Michael Carr, September 10, 1991

Biogeochemical Information About Mars Inferred from SNC Meteorites, presentation by James L. Gooding, Code SN/Solar System Exploration Division, NASA Johnson Space Center, September 10, 1991

Mars Soil Chemistry, presentation by Benton Clark, Martin Marietta, Denver, Colorado, September 10, 1991

Stability of Water and Ice on Mars, presentation by Bruce M. Jakosky, Laboratory for Atmospheric and Space Physics, University of Colorado, September 10, 1991

UV and Radiation Stress, presentation by Richard B. Setlow, Brookhaven National Laboratory, September 10, 1991

Sporulation and Resting Forms and the Survival Capacity, written presentation by Philipp Gerhardt, Michigan State University, September 10, 1991

Detection of Nucleic Acids and Citations Indicating the Inadequacy of Microscopic and Culture Methods for Describing Microbial Communities, presentation by Thomas Schmidt, September 10, 1991

Scientific and Technological Issues Associated with Sample Return Missions, presentation by Roger D. Bourke, Manager, Exploration Initiative Studies Office, Jet Propulsion Laboratory, September 11, 1991

Alternative Sterilization Methods, presentation by Robert Howell, Bionetics Corporation, September 11, 1991

Societal Issues Associated with Planetary Protection and Back Contamination, presentation by Margaret Race, University of California, Berkeley, September 11, 1991

Exobiology and Planetary Protection—The Evolving Law, presentation by George S. Robinson, Smithsonian Institution, September 11, 1991

Papers Cited

"Action Spectra for Survival and Spore Photoproduct Formation of *Bacillus subtilis* Irradiated with Short-Wavelength (200-300 nm) UV at Atmospheric Pressure and *in Vacuo*" (*J. Photochem. Photobiol. B:Biol.*11:69-80, June 7, 1991) C. Lindberg and G. Horneck, Köln, Germany

"Can Spores Survive in Interstellar Space?" (*Nature* 316:403-407, August 1985), Peter Weber and Mayo Greenberg, Laboratory of Astrophysics, University of Leiden, 2313 A.V. Leiden, The Netherlands

"Ejection of Rock Fragments from Planetary Bodies" (*Geology*, pp. 144-148, February 1985), H. Jay Melosh, Lunary and Planetary Laboratory, University of Arizona

"Evolutional and Ecological Implications of the Properties of Deep-Sea Barophilic Bacteria" (*Proc. Natl. Acad. Sci. USA* 83:9542-9546, December 1986), A. Aristides Yayanos, Scripps Institution of Oceanography, University of California

"First Biological and Dosimetric Results of the Free Flyer Biostack Experiment A0015 on LDEF," G. Reitz, H. Bucker, R. Facius, G. Horneck, M. Schafer, and J.U. Schott, DLR, FF-ME, Biophysics Division, Linder Hohe, 5000 Köln 90, Germany

"Ionizing Radiation at the Surface of Mars" (unpublished paper), James L. Gooding and Gautaum D. Badhwar, September 10, 1991

"The Large Crater Origin of SNC Meteorites" (*Science* 237:738-743, August 14, 1987), A.M. Vickery and H.J. Melosh

"Long-Term Exposure of Bacterial Spores to Space" (submitted to *Nature*), G. Horneck, H. Bucker, G. Reitz, Institute for Aerospace Medicine, DLR, Linder Hohe, 5000 Köln 90, Germany

"The Rocky Road to Panspermia" (*Nature* 332:6166, April 21, 1988), Jay Melosh, Lunar and Planetary Laboratory, University of Arizona

"Spore Thermoresistance Mechanisms" (Chapter 2 from *Regulation of Procaryotic Development*, American Society for Microbiology, Washington, D.C., 1989), Philipp Gerhardt and Robert E. Marquis

Other Resource Materials

DeVincenzi, D.L., H.P. Klein, and J. Bagby. 1991. *Planetary Protection Issues and Future Mars Missions.* NASA Ames Research Center, Moffett Field, Calif.

"European Space Report," *Policy Issues in East and West,* Volume 2, Number 14, n.d.

Excerpts from *Recommendations on Quarantine Policy for Mars, Jupiter, Saturn, Uranus, Neptune, and Titan,* Committee on Planetary Biology and Chemical Evolution, Space Science Board, National Research Council, National Academy of Sciences, Washington, D.C., 1978

Klein, H.P. 1991. *Planetary Protection Issues for the MESUR Mission: Probability of Growth (P_g).* NASA conference publication. NASA Ames Research Center, Moffett Field, Calif.

"Mars Environmental Survey (MESUR)," NASA informational brochure, n.d.

"Marsnet—A Mars Network of Surface Stations" (unpublished white paper), G.E.N., Scoon, ESA/ESTEC, n.d.

Mazur, Peter. 1980. "Limits to Life at Low Temperatures and at Reduced Water Contents and Water Activities." *Origins Life* 10:137-159.

Revised Planetary Protection Policy, presentation by D. DeVincenzi to Space Science Board, October 10, 1985

Spaceflight Environmental Effects Newsletter, Special Issue, Volume 1, Number 5, August 10, 1990

"The Viking Mission and the Question of Life on Mars," *Journal of Molecular Evolution*, 14, Numbers 1-3, 1979

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Excerpts from the 1978 Report*

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Recommendations on Quarantine Policy for Mars Based on the Current Viking Findings

The current NASA policy on the likelihood of growth of terrestrial microorganisms on Mars is based on the December 14, 1970, Space Science Board report, *Review of Sterilization Parameter Probability of Growth (P_g)*.

The report established the minimum conditions necessary to define a microenvironment on Mars that would support growth of the most "hardy terrestrial organisms." The conditions established were the following:

(a) Water activity (a_w) $\geqslant 0.95$.

(b) Temperature $\geqslant 0°C$ for at least 0.5 h/day.

(c) Nutrients: At least small amounts of water-soluble nitrogen, sulfur, phosphorus, carbon (and/or light). pH values between 5 and 8.

(d) Attenuation of uv flux by more than 10^3.

(e) Antinutrients—absence of antimetabolites.

All the above conditions must occur simultaneously, or nearly so.

The report then proceeded to estimate the value of P_g, the "estimated probability that growth and spreading of terrestrial organisms on the planet surface will occur." The estimated value of P_g was 3×10^{-9}, with less than one chance in a thousand that it exceeded 1×10^{-4}. For the Viking project, NASA adopted a value of $P_g = 10^{-6}$, some three orders of magnitude more favorable to growth than the best estimate of the review committee, but still two orders of magnitude less than the extreme upper limit. The adoption of this

value required terminal heat sterilization of the entire Viking Lander but not of the Orbiter. The value remains NASA policy to date.

I. VIKING FINDINGS PERTINENT TO QUARANTINE

Estimating the likelihood of the growth of terrestrial organisms on Mars requires a comparison between the known physical and chemical limits to terrestrial growth and the known and inferred conditions present on or just below the Martian surface. Table 1 makes that comparison in abbreviated form. Appendix A discusses in fuller form the inferences that can be drawn from the Viking findings about those physical and chemical characteristics of the Martian surface that are pertinent to the question of the growth of terrestrial microorganisms.

Orbital measurements have covered appreciable fractions of the planet's surface, but the two Landers (VL-1 and VL-2) have sampled only a few square meters of the surface at two subpolar sites. The biologically relevant experiments were conducted on soil samples acquired during the Martian summer and early fall from as deep as 6 cm below the surface. (In March 1977 a sample was acquired from a depth of 20 cm, but as of April 1977 an inorganic analysis is the only experiment that has been performed.) Nevertheless, certain extrapolations relevant to the quarantine question can be made with various degrees of confidence to other regions of the planet, to greater depths, and to other seasons of the year.

TABLE 1   Limits for Growth of Terrestrial Organisms

Factor	1970 Study	Refs. 2 and 3	Conditions on Mars[a]
Water activity (a_w)	≥0.95	>0.9	0 to 1
Water (liquid)	—	Required	Not detected
Temperature	≥0°C	>−15°C	+20 to −143°C (see text)
pH	5–8	<11.5	Not known
Ultraviolet radiation[b]		0.1 J cm^{-2}	0.04 J cm^{-2} min^{-1}
Ionizing radiation[b]	—	2–4 Mrad	<500 rad/yr[c]
Nutrients	See text and Refs. 2 and 3		Organic compounds ≤ppb; most required elements detected (see text)[d]
Antimetabolites	None present		Strong oxidants present (see text)[d]

[a]Cf. Reference 1; uv flux data from Reference 18.
[b]Limit for survival. Limits for growth are not known.
[c]See p. 11.
[d]At VL-1 and VL-2 sites.

II. CONCLUSIONS ON THE LIKELIHOOD OF THE GROWTH OF TERRESTRIAL ORGANISMS ON MARS

We turn now to a reassessment of P_g, the likelihood of the growth of terrestrial organisms on Mars. We will consider three regions separately: (1) subpolar areas within a few centimeters of the surface, (2) subpolar regions more than a few centimeters below the surface, and (3) the residual polar caps. Finally, we will discuss briefly the likelihood that terrestrial organisms could survive transport at or above the surface from one region to another.

A. Subpolar Regions within about 6 Centimeters of the Surface

Our conclusion is that no terrestrial organisms could grow within a few centimeters of the surface in the regions lying between the two residual polar caps. We base this judgment on the following of the Viking findings:

— The presence in VL-1 and VL-2 sample of strong oxidants.

— The absence of detectable organic compounds, which (a) attests to the power of the oxidants and (b) renders unlikely the existence of the specific types of organic compounds required for terrestrial heterotrophic organisms.

— The inability of physical shielding by a rock to eliminate the oxidants.

Our conclusion is reinforced by three additional factors that were well known before the mission:

— The unlikelihood of organisms being deposited in regions that receive sufficient visible light to support photosynthetic autotrophy without at the same time receiving lethal fluxes of ultraviolet radiation.*

— The exceedingly low probability for the existence of liquid water with activity (a_w) high enough to support terrestrial growth.

— The fact that, even if liquid water were present, vegetative cells would be subjected to daily cycles of injurious freezing; and only vegetative cells can grow.

*Although unlikely, the probability is not zero. Sagan and Pollack[10] have calculated that, although the uv flux is attenuated several millionfold at 0.8 cm below the Martian surface, the flux of visible light would still be 3.8×10^2 erg cm^{-2} sec^{-1} at that depth.

It is highly likely that the surface conditions enumerated above at the VL-1 and VL-2 sites prevail over the subpolar regions of the planet. This conclusion is based on

1. The similarity in the findings at two widely separated points for the elemental composition of the regolith and for the results of the organic analysis and the gas-exchange experiments.

2. The strong probability that the oxidants are derived from atmospheric reactions or atmosphere–regolith reactions. Accordingly, it is difficult to conceive of regions that would be accessible to terrestrial microorganisms and at the same time be capable of excluding the atmosphere.

3. The fact that the Infrared Thermal Mapper (IRTM) has mapped a sizable fraction of the Martian surface without detecting thermal heterogeneities significantly more favorable to terrestrial growth than those that we have reviewed in Appendix A.

Viking has provided much information that was either not known beforehand or was known only with considerable uncertainty. None of this new information suggests that the Martian surface is less harsh to terrestrial microorganisms than was thought prior to Viking.* On the other hand, two pieces of information indicate that it is harsher than was thought previously: the lack of detectable organic compounds and the presence of strong oxidants even in regions physically shielded from uv.

Our conclusion is that no terrestrial organism could grow under the conditions found by Viking to prevail on subpolar surfaces at the landing sites and none could grow under the conditions that are highly likely to prevail throughout the entire subpolar region. Few if any terrestrial organisms could grow in contact with even one of the adverse conditions cited, much less grow when exposed to all of them simultaneously. Although we cannot absolutely rule out the existence of oases capable of supporting terrestrial life, we believe, for the reasons cited, that the likelihood of their existence is extremely low.

Unfortunately, we know of no quantitative basis for assigning a numerical probability to "extremely low" when no oasis has been

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*The demonstration by Viking that the atmosphere contains nitrogen answers an important question that was unknown previously. However, the ignorance prior to Viking of the existence of nitrogen was not a significant factor in prior estimates of the probability of growth of terrestrial organisms.

detected and when the weight of evidence is that none can exist. And yet a numerical value for P_g is required in order to determine what procedures are needed to reduce the microbial burden on future spacecraft to Mars to levels that fulfill current COSPAR quarantine policy. Reluctantly, then, *we recommend for these purposes, and these purposes alone, that NASA adopt a value of* $P_g < 10^{-10}$ *for the subpolar regions of the planet within 6 cm of the surface.** This number, which is more than four orders of magnitude below the current value of P_g, reflects the fact that Viking has found the conditions to be considerably harsher to terrestrial life than was heretofore assumed and has obtained evidence that renders the existence of oases far less likely than was heretofore assumed.

B. Regions More than 6 Centimeters below the Surface of Subpolar Regions

As mentioned, Viking conducted biology experiments and organic analysis on samples obtained from depths of 4–6 cm. Greater depths would be required to reduce or eliminate the lethal surface conditions. The depths required are unknown chiefly because the relation between depth and the presence of oxidants is unknown. However, the maximum temperature falls rapidly with depth. In the northern hemisphere, even at a depth of 4 cm, the maximum temperature is estimated to be 20° below the minimum confirmed growth temperatures (–15°) observed for terrestrial organisms (Appendix B). By a depth of 24 cm, the *maximum* temperature is estimated to be –50°C, some 35° below the minimum confirmed terrestrial growth temperature. In the southern hemisphere, the *maximum* temperature at a depth of 24 cm is estimated to be –35°C, still 20° below the minimum terrestrial growth temperature.[4,8]

At increased depths there is an increased likelihood of encountering ice, the existence of which would enhance the possibility of liquid water. But water that is liquid below –20°C and is in equilibrium with ice has an activity (a_w) below that which will support the

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*We obtain this value by estimating probabilities of $<10^{-2}$ for the presence of liquid water of high enough a_w, $<10^{-1}$ for the ability to survive multiple freezing and thawing, $<10^{-1}$ for the avoidance of lethal uv, $\ll 10^{-2}$ for the presence of organic compounds of appropriate types in appropriate concentrations, $\ll 10^{-3}$ for the absence of powerful oxidants, and 0.1 that the deposited microorganism is an anaerobe.

growth of any known terrestrial organism capable of growing under the partial pressure of oxygen on Mars (Appendix B, Figure B.2).[9]

Thus, temperature alone seems an absolute barrier to the growth of any terrestrial organisms at depths below a few tenths of a meter. But again, sufficient uncertainties exist to preclude an absolute statement to this effect; viz.,

— Although the surface temperatures are derived directly from the orbital infrared measurements and are consistent with the direct meteorological measurements at the landing sites 1.5 m above the surface, the estimates of subsurface temperatures require assumptions about the thermal diffusivity of the soil. The range of error is estimated by Kieffer[8] to be 5°C. This error would not be sufficient to change our conclusions, but larger errors are conceivable.

— There could exist heterogeneities below the resolving power of the IRTM (a minimum of 2 km) that have higher temperatures.

— Although there is extensive information on the minimum growth temperatures of terrestrial microorganisms, the remote possibility exists that some unknown organism has a growth minimum below -15°C. We view this as extremely remote because, as indicated in Appendix B, the number of species capable of growth diminishes drastically as the temperature is lowered below 0°C. Furthermore, growth below -15°C is tantamount to growth in $\geqslant 8$ osmolal solute, conditions that even at ordinary temperatures preclude the growth of all except halophiles and osmophiles.

— There is the remote possibility that there exists somewhere a narrow zone of subsurface that is deep enough to preclude oxidants and shallow enough to have temperatures high enough to support growth.

Although these uncertainties prevent us from concluding that the possibility for growth is zero, we are still forced to conclude that subsurfaces of Mars are exceedingly harsh for terrestrial life. Accordingly, *for the specific purpose of determining quarantine requirements for future Martian missions, we recommend that NASA adopt a value of $P_g < 10^{-8}$ for subsurfaces in the subpolar regions of the planet.*

C. The Residual Polar Caps

The arguments just presented for subsurface regions generally apply to the residual polar caps as well. As in the subsurface regions, the

temperatures mapped by the IRTM are too low to permit the growth of known terrestrial organisms. However, thermal heterogeneities have been detected. The maximum temperatures observed (237 K) are not high enough to permit the growth of earth organisms, but their presence raises the remote possibility that there exist other undetected heterogeneities for which the temperature does rise high enough. But warmer regions will also be drier regions, because the increased vapor pressure associated with higher temperatures would cause water to distill rapidly from these regions and freeze out at the cold trap furnished by the remainder of the residual cap.[4] The water ice itself in the residual caps constitutes a possible source of liquid water, provided that special conditions were present to permit that ice to liquefy rather than to sublime (e.g., freezing point depression by electrolytes). But even then, as in the case of subsurfaces, the temperatures would be too low to permit the growth of terrestrial organisms.

The polar regions would not be immune from the atmospheric oxidants, but chemical interactions between atmosphere and ice might be different from chemical interactions between atmosphere and regolith.

Our conclusions about the likelihood of growth in the residual polar caps are similar to those reached in Section B above for subsurface subpolar regions—it is extremely low. Nevertheless, because there is more uncertainty about the physical and chemical conditions at the residual polar caps, we believe that these regions should be handled with prudence and *recommend that they be assigned a value of* $P_g < 10^{-7}$.

D. Transport from Subpolar Regions into the Residual Polar Caps or into Putative Oases

There is little likelihood that any terrestrial organism could survive a voyage on or above the surface requiring more than a few minutes. First, the uv flux on the surface of Mars is 4×10^{-2} J cm^{-2} min^{-1}, and that flux would kill the most resistant of terrestrial microorganisms in a few minutes (upper terrestrial limit 0.1 J/cm^2) (Table 1). Second, organisms protected from the direct exposure to the uv by a layer of soil particles would nevertheless be in contact with the oxidants in those soil particles.

One consequence of these lethal conditions is that our recommended value of $<10^{-7}$ for P_g in the residual polar caps applies only

C̸ 2

to terrestrial organisms that are released directly in that region. The P_g for organisms transported into the polar caps from the subpolar regions would be orders of magnitude lower. Similarly, even if Mars were to possess oases that were hospitable to terrestrial life, few if any terrestrial organisms would survive a surface or aerial trip to the oasis and few if any would ever survive an escape from the oasis.

III. LIMITS TO THE GROWTH OF TERRESTRIAL LIFE VERSUS THE QUESTION OF INDIGENOUS LIFE ON MARS

The evidence that leads us to the conclusion that terrestrial micro-organisms have little and in most regions of the planet no probability of growth does not rule out the possibility that indigenous life forms may exist currently on Mars or may have existed sometime in the past. The limiting conditions listed in Table 2 for terrestrial life are not the limits for conceivable life elsewhere.

There is fairly wide agreement that life, if it exists elsewhere, is based on carbon chemistry and that it requires nitrogen; organic compounds of high information content, energy, and substrates to permit the synthesis of the organic compounds; and liquid water. Although, as discussed, organic compounds and liquid water have not been detected on Mars, there is no basis for precluding their existence. There is, moreover, strong evidence that liquid water in large quantities existed in the Martian past.

It might be argued that, if indigenous life forms do exist, they themselves could constitute micro-oases for the growth of terrestrial organisms. We consider this unlikely. For example, a Martian organism growing in thermal equilibrium with its surroundings at $-40°C$ would be of no value to a terrestrial organism incapable of growing below $0°C$. A Martian organism that maintains its temperature at $0°C$ even when the external temperature is $-40°C$ is conceivable. However, to do so, a spherical organism 2×10^{-4} cm in diameter, for example, encased in efficient insulation $\geqslant 1$ mm thick would have to assimilate and burn about 1000 times its steady-state concentration of organic compounds *per second* to maintain the 40-degree differential. The problem would be only slightly less serious in a macroscopic Martian organism. Analogous difficulties arise in postulating that the organic compounds in putative Martian biota would be compatible with and utilizable by the enzyme systems of terrestrial microorganisms.

TABLE 2 Estimated Contributions to P_g for Jupiter and Saturn

Factor	1974 Jupiter Report	1976 Uranus Report	Comments
Temperature	1	1	Assumed between -20 to 100°C
Pressure	1	1	Not a critical parameter for microbiology
Radiation	1	Not specified but <1	Deleterious
Liquid H_2O	1	1	Assumed
Nutrients	10^{-1}	$<10^{-3}$ [a]	Organics, ions = aqueous solution
Anaerobiosis	10^{-1}	10^{-1}	About 0.10 of the earth's microbes are anaerobes, but these are unlikely to be spacecraft contaminants
NH_3 toxicity	10^{-2}	$<10^{-4}$ [b]	
Growth in aerosols	Not specified	$<10^{-3}$ [a]	Completion of life cycle in the atmosphere has never been reported for any earth organisms
Convection to lethal temperatures	10^{-3}	$<10^{-3}$	All models predict that organisms will be carried from water levels to lethal depths; the times required are somewhat model dependent
TOTALS	10^{-7}	$<10^{-14}$	

[a] Based on more detailed analyses.[2,3]
[b] New information, e.g., Reference 22.

IV. CONCLUSIONS PERTINENT TO THE CURRENT VIKING ORBITERS

As of August 1977, two years have elapsed since the unsterilized Orbiters were launched from earth. Any organisms on the outer surface of the Orbiter have surely been killed by uv irradiation. Most organisms in the interior of the Orbiter have been subjected to moderate temperatures (10 to 38°C), high vacuum, and some ionizing radiation.[11] Although the cell dehydration associated with the high

vacuum would be lethal to a fraction of the microbial population, many (perhaps 1 to 10 percent) would likely survive.[6,12,13] Some protons from galactic cosmic rays and solar flares would strike organisms in the interior, but the dose would be appreciably less than 500 rad/year,[11,14] and many microorganisms can survive such doses. (The flux of solar protons far exceeds that from galactic source, but the great bulk of the solar protons have energies of $\leqslant 1$ MeV,[11] and such protons are only capable of penetrating $\leqslant 0.1$ mm of material with a density of 1, e.g., water.[14]) Conservatively, then, one cannot assume that the microbial burden within the Orbiter has decreased by more than 1 or 2 orders of magnitude since launch.

In spite of the expected survival of a fraction of the original burden of terrestrial microorganisms, our new estimates of the values of P_g lead to the conclusion that COSPAR requirements for planetary quarantine will not be compromised by lowering the periapsis of the Orbiters to 300 km. Indeed, with the new values for P_g, still lower periapses for unsterilized Martian orbiters may well be compatible with COSPAR requirements. NASA will probably wish to determine these minimum orbital altitudes before assessing and designing Mars follow-on missions in detail.

V. QUARANTINE STRATEGY FOR FUTURE MISSIONS TO THE MARTIAN SURFACE

Our Committee has recommended that the next phase in the biological exploration of Mars should be to acquire and characterize soil samples from areas likely to contain sediments and ice–regolith interfaces.[1] Locating these areas and locating sites that are shielded from the powerful atmospheric ultraviolet radiation and the powerful surface oxidants will require subsurface sampling by a soft lander, by penetrators, or by both. The samples acquired from the subsurface of Mars should be characterized with respect to organic compounds, carbon and sulfur isotope ratios, the amount and state of water, the presence of water-soluble electrolytes, and the existence of non-equilibrium gas compositions. The greater the extent to which samples possess these characteristics the greater the priority for the initiation of a second phase of post-Viking biological exploration of Mars—a detailed search for evidence of present or past life on Martian samples returned to earth.

With respect to quarantine considerations for the mission that conducts the first exploratory phase, our estimates for the values of

P_g lead to the conclusion that terminal heat sterilization would not be required in the case of a nominal soft landing in the subpolar regions (Section A) and possibly in other cases as well. However, we would have no objections to sterilization *provided that* it has no impact on the scientific payload of the landers and that it does not increase the mission cost. (We have been informed by representatives of NASA that this may be the case.) Decisions on scientific payloads for the missions should be based on their scientific quality and cost effectiveness. *We would object to the elimination of an experiment or the degradation of its performance because of the imposition of unessential sterilization requirements.*

In the report *Post-Viking Biological Investigations of Mars*,[1] we stated that we consider metabolic-type life-detection experiments on the surface of Mars to be of low priority scientifically. Nevertheless, NASA may decide to include them. If so, a limiting factor with respect to the allowable microbial burden on a soft lander would likely become the avoidance of contaminating the metabolic experiment by terrestrial microorganisms.

Appendix A:
Findings from Viking Pertinent to the
Possible Growth of Terrestrial
Microorganisms on Mars*

I. DEFINITIVE FINDINGS FROM VIKING

A. Water

The gas chromatograph–mass spectrometer (GCMS) has detected less than 0.1 percent water in soil samples (several tenths of a percent in one sample collected from beneath a rock). The current belief is that this water represents mineral hydrate water of moderate or low thermal stability. Neither this instrument nor the others on the lander were designed to detect free liquid water, nor have they done so.

Unfortunately, Viking carried no instrument to measure relative humidity. However, indirect evidence (e.g., cloud formation) indicates that saturation does occur in the atmosphere.

The probability for the existence of liquid water anywhere on the planet remains low. The surface temperatures and atmospheric pressures preclude the existence of pure bulk liquid water under equilibrium conditions. However, there continue to be three remote possibilities for the existence of liquid water: (1) water adsorbed to subsoil, (2) water that is liquid by virtue of kinetic factors slowing the approach to equilibrium (i.e., conditions under which diffusion of water is slower than diffusion of heat), and (3) water that has its chemical potential (and hence freezing point) lowered by the presence of dissolved solutes. The solutes could be one or more of the several salts that are almost certainly present. The eutectic points of

*Pertinent references not cited here will be found in Reference 1.

salts like $CaCl_2$ $MgCl_2$, and K_2CO_3 are below $-30°C$; hence their presence would permit stable liquid water down to these temperatures. The electrolyte concentrations, however, would be multimolar.

Another argument against the existence of liquid water at the landing sites is the findings of the Labelled Release and Gas Exchange biology experiments. In both cases, the initial addition of water vapor or liquid water to the soil samples dissipated the reactants so that further additions produced no further reactions (release of $^{14}CO_2$ and release of oxygen in the two experiments, respectively). Presumably, therefore, no reactions at all would have been observed if the soil itself had been exposed to high-activity liquid water just prior to the acquisition of samples by the Landers.

B. Temperature

The maximum temperatures observed at the surface of the landing sites during the summer–autumn observation period were -2 to $-3°C$. This is below the minimum growth temperature of most terrestrial microorganisms, although, as discussed later and in Appendix B, a few terrestrial organisms can grow at temperatures as low as $-14°C$. In the southern hemisphere of Mars, the maximum summer surface temperatures may reach $20°C$.[4]

At night, even in summer, the temperature drops to $\leqslant-83°C$. Dry bacterial and fungal spores could survive many cycles of such freezing, but hydrated and germinated spores or vegetative cells of most terrestrial species could not.[5-7] And any terrestrial microorganism that is to grow on Mars must by definition be in the vegetative state to carry out such growth.

C. Lack of Detected Organic Compounds

No organic compounds other than traces attributable to terrestrial contaminants have been detected in regolith samples analyzed by the GCMS. If volatizable organic compounds were present in the samples, they were either present in concentrations below the parts per billion range (the detection limit of the instrument) or they were totally restricted to substances like methane with molecular weights of less than 18, which were undetectable or detectable only at reduced sensitivities.

The inability to detect organic compounds does not, of course, prove that none was present. But even if trace amounts of organic

compounds are in fact present in the soil of the landing sites, the probability is remote that these would provide a nutrient medium that could be used by terrestrial microorganisms (see Reference 3 for further discussion).

D. Elemental

The biologically vital element nitrogen has now been shown to be in the Martian atmosphere. Calcium, sulfur, magnesium, chlorine, and probably potassium and phosphorus have also been detected in soil samples. All six are essential to terrestrial living systems. Instrument limitations precluded the detection of sodium, but there is no reason to believe that it is not present, although probably only in low concentrations.

One striking finding is that the elemental composition of the samples was nearly identical at the two widely separated landing sites.[26] This similarity indicates that the fine-grained material in at least the upper surfaces of the regolith has been thoroughly mixed over large regions of the planet—presumably as the result of wind action.[27]

E. Oxidants

Two lines of evidence indicate that strong oxidants are present in at least the top few centimeters of the regolith at the landing sites. The first line of evidence comes from orbital measurements of the atmosphere and from modeling. One model predicts the existence of active strongly oxidizing species, especially hydrogen peroxide. Second, the gas-exchange experiment (GEX) on the Viking landers showed the release of up to nearly a micromole of oxygen when samples were humidified with water and warmed to -10°C.

The GEX experiment suggests that oxidants are present to at least the 4–6-cm depth from which samples were acquired. The experiment also showed that the oxidants were present in samples collected from beneath a rock, a rock that presumably had laid undisturbed for many years. Finally, the experiment showed that the oxidants were present at both landing sites.[28]

The oxidants are believed to be responsible for the lack of detectable organic compounds, i.e., they have decomposed them.

II. EXTRAPOLATION FROM THE VIKING FINDINGS TO THE PLANET'S SURFACE AS A WHOLE, TO REGIONS BELOW THE SURFACE, AND TO OTHER SEASONS OF THE YEAR

Surface temperatures in the Martian winter will drop far lower than those experienced during the Lander experiments. Estimates from the infrared thermal mapper (IRTM) indicate that the *maximum* surface temperature will fall below -15°C (the minimal terrestrial growth temperature—see Appendix B) for more than half the Martian year at the VL-2 site (48° N) and further north. At VL-1 (22° N) the maximum surface temperature will just about reach -15°C in the winter.[8] (Orbital IRTM measurements during winter will become available during the ensuing months.)

In considering extrapolations from the findings of VL-1 and VL-2 on surface chemistry, we note that, although the two landing sites (22° N and 48° N) are separated by some 1500 km in latitude and 176 degrees in longitude, the results of the gas-exchange (GEX) and labeled release (LR) biology experiments and of the organic and inorganic analyses at the two sites were either similar or essentially identical.* Strong similarities were evident as well from the imaging experiment and from the atmospheric analyses. As noted, the results of the GEX, LR, and GCMS experiments are consistent with the presence of powerful oxidants in the surface samples. Since these oxidants are almost certainly derived from atmospheric photochemical reactions or from chemical reactions between atmospheric species and the regolith, there is every reason to expect that they will be globally distributed in the Martian surface, except possibly in the residual polar caps.

Certain extrapolation can also be made to depths below the 4-6 cm sampled by the Landers.

A. Water

Several Viking experiments have confirmed or strengthened the inference that large amounts of water are locked beneath the surface in the form of ice. Subsurface liquid water is conceivable; however,

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*Samples from the two sites in the Pyrolytic Release (PR) experiment, however, responded differently to the addition of water vapor.[25] The experimenters suggest that this reflects differences in the properties of the soil at the two sites, but they draw no inferences as to the nature and degree of the differences.

because of the low temperatures at subsurfaces (see below), the existence of liquid water in an equilibrium state would require multimolar concentrations of electrolytes (see Appendix B, Table B.1).

B. Temperature

The maximum summer temperatures some 6 cm below the surface at the VL-1 and VL-2 sites are estimated from the IRTM measurements to be -35°C.[8] This temperature is 20° *below* the minimum confirmed growth temperature for terrestrial microorganisms. It is even below the lowest growth temperature ever claimed in published reports. At a depth of 24 cm, the maximum summer temperatures at the VL-1 and VL-2 sites are estimated to be -50°C, or 35° below the minimum confirmed terrestrial growth temperature. In the southern hemisphere as a result of the eccentricity of the Martian orbit, the maximum surface temperatures between latitudes 5° and 45° are about 15° warmer than at the present landing sites. As a result, at subsurface depths sufficient to damp out diurnal variations, the maximum summer temperature is calculated to be about -35°C, still some 20° below the minimum confirmed terrestrial growth temperature.[4]

C. Ultraviolet Light

As shown in Table 1, the flux of ultraviolet radiation impinging on the Martian surface would be rapidly lethal to any terrestrial organism. However, the uv flux is sharply attenuated below the surface. For example, Sagan and Pollack[10] estimate an attenuation of several millionfold at a depth of 0.8 cm.

D. Oxidants and Organic Compounds

Since the oxidants in the regolith are almost certainly derived from atmospheric processes, their concentrations ought to diminish with depth below the surface. But the relationship between depth and concentration is unknown. Presumably at least some of the oxidant species are diffusable, for they were present in the soil samples collected from beneath the rock at the VL-2 site.

Since the lack of detectable organic compounds within 4–6 cm of the surface seems due to the presence of the oxidants, the likelihood of organic compounds ought to increase with depth. (Organic matter

must be present at least transiently on the Martian surface, if from no other source than the infall of carbonaceous chondrites.)

Although the Martian surface is strikingly similar at two widely separated points when viewed close-up from the two Landers, the surface is strikingly heterogeneous when viewed from orbit. Still, there is no evidence that any of the heterogeneities represent oases that possess characteristics more favorable to terrestrial life than those already enumerated. One dramatic class of heterogeneities, for example, is the huge channels that were almost certainly formed by flowing liquid water. But these channels are too old (probably $\geqslant 10^9$ years) to have much bearing on their current suitability for the growth of terrestrial organisms, except that they might possibly contain concentrated deposits of electrolytes and organic compounds.

The orbital infrared temperature and water-vapor measurements also show heterogeneities, but again none of those detected have properties significantly more favorable to terrestrial life than do the larger-scale features. The resolving power of the IRTM is 0.3°, which translates to 8 km at the normal periapsis of 1500 km and 1.6 km for the now lowered periapsis of Orbiter 1 (300 km). Smaller oases with respect to some of the biologically relevant factors are conceivable (e.g., higher temperatures on south-facing slopes in the northern hemisphere; higher temperatures because of heat absorbed by dark objects). It is difficult, however, to conceive of any oasis on the surface of subpolar regions that would be accessible to terrestrial organisms and yet not contain the atmospherically induced oxidants. As mentioned, the subrock sample at VL-2 indicates that some of the oxidants can diffuse in the regolith.

Appendix B
Minimum Temperature for
Terrestrial Microbial Growth

The most thorough review known to us of the minimum growth temperatures of terrestrial microorganisms is that of Michener and Elliott.[15] A histogram summarizing their findings on reports of growth below 0°C is shown in Figure B.1. Many of these reports are based on incubation times of over a year. We separate bacteria from fungi because the latter are nearly all aerobic and would be incapable of growing at Martian partial pressures of oxygen. The single case of a bacterium growing below -12°C was a report of growth at -20°C. Neither it nor the three reports of fungal growth below -12°C have been confirmed. Michener and Elliott point out that "The best evidence that growth does not generally occur in foods in this temperature range [i.e., <-17°C] is that billions of cartons of frozen food have been stored at or near -18°C without reported microbial spoilage."

A more recent study by Fennema *et al.*[16] confirms Michener and Elliott's conclusion that microbial growth in foods does not occur at -18°C.

This inability of organisms to grow below about -15°C is consistent with the known physical state of aqueous solutions at these temperatures. As Table B.1 shows, when solutions of sodium chloride in water, for example, are equilibrated at various subzero temperatures, the concentrations in the unfrozen portions exceed 4 molal below -15°C. For solutes in general, the concentrations of solutes in the unfrozen portions of solutions are given by $\phi \nu m = \Delta T/1.86$ where ϕ is the osmotic coefficient, ν the number of species into which the solute dissociates, and m is the molality.[17] Aside from the toxic effects to nearly all microorganisms of such high concentrations of electrolytes, the high concentrations also depress the water
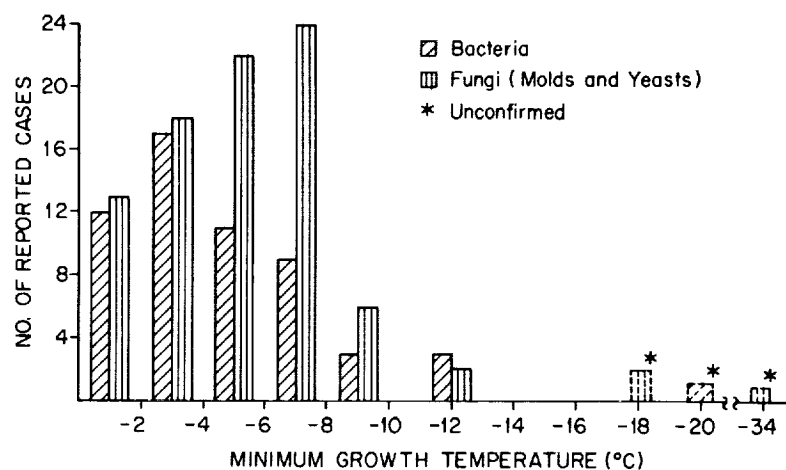
FIGURE B.1   Reported cases of microbial growth below 0°C. (Adapted from Reference 15.)

TABLE B.1   Solute Concentrations and Water Activities in NaCl Solutions at Various Temperatures

Temperature (°C)	Concentration NaCl[a] (molal)	$a_w{}^b$
-5	1.45	0.95
-10	2.79	0.91
-14	3.73	0.87
-15	3.96	0.86
-16	4.17	0.85
-18	4.58	0.84
-20	5.00	0.82

[a]From Reference 19.

[b]$a_w = p_{H_2O}$ (solution)$/p_{H_2O}$ (liquid, pure)

$\equiv p_{ice}/p_{H_2O}$ (liquid pure).

Calculated from data in Reference 20. See Figure B.2.
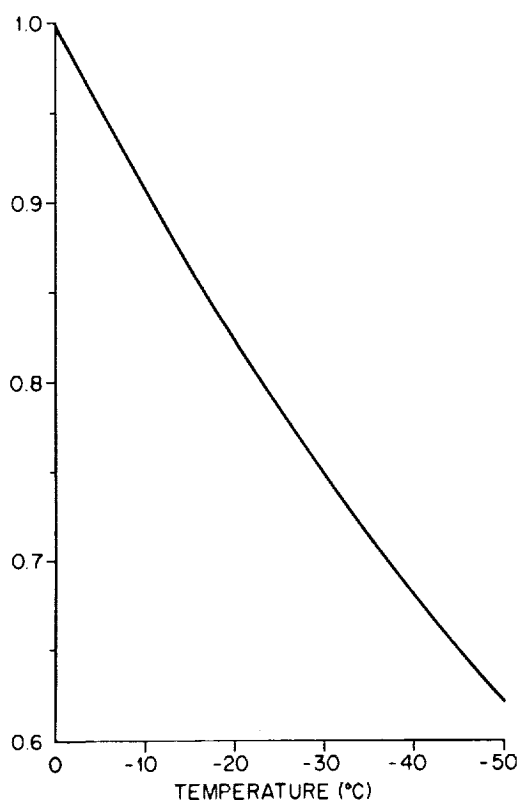
FIGURE B.2 Water activity $a_w = p_{ice}/p_{H_2O}$ of a solution in equilibrium with ice as a function of temperature.

activity (a_w) below the value permitting the growth even at optimal temperatures of all microorganisms save halophilic and osmophilic forms. As shown in Table B-1 and Figure B-2, the values of a_w at -14, -16, -18, and $-20°C$ are 0.87, 0.85, 0.84, and 0.82, respectively.

E

Additional Related Information

TABLE E.1 Proposed Planet/Mission Categories and Range of Requirements

	Category I	Category II	Category III	Category IV	Category V
Type of mission	Any but Earth return	Any but Earth return	No direct contact (flyby, some orbiters)	Direct contact (lander, probe, some orbiters)	Earth return
Target planet	Sun, Mercury, Pluto	To be determined	To be determined	To be determined	To be determined
Degree of concern	None	Record of planned impact probability and contamination control measures	Limit on impact probability Passive bioload control	Limit on probability of nominal impact Limit of bioload (active control)	If *not* safe for Earth return: —No impact on Earth or Moon —Sterilization of returned hardware —Containment of any sample

Representative range of requirements	None	—Documentation only (all brief): • PP plan • Prelaunch report • Postlaunch report • Postencounter report • End-of-mission report	—Documentation (more involved than Category II): • Contamination control • Organic inventory (as necessary) —Implementing procedures such as: • Trajectory biasing • Cleanroom • Bioload reduction (as necessary)	—Detailed documentation (substantially more involved than Category III): • P_c analysis plan • Microbial reduction plan • Microbial assay plan • Organics inventory —Implementing procedures such as: • Trajectory biasing • Cleanroom • Bioload reduction • Partial sterilization	*Outbound* —Per category of target planet/ outbound mission *Inbound* If *not* safe for Earth return: —All of Category IV requirements —Continual monitoring of project activities —Preproject advanced studies/research If safe for Earth return: None

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NASA MANAGEMENT INSTRUCTION
NMI 8020.7B

April 17, 1991   NMI 8020.7B

NASA		NMI 8020.7B
Management	Effective Date:	April 17, 1991
Instruction	Expiration Date:	April 17, 1995

RESPONSIBLE OFFICE: S/Office of Space Science and Applications

SUBJECT: BIOLOGICAL CONTAMINATION CONTROL FOR OUTBOUND AND
           INBOUND PLANETARY SPACECRAFT

1.   PURPOSE

     This revised Instruction establishes NASA policy and assigns
     responsibility for preserving solar system conditions for
     future biological and organic constituent exploration and
     for protecting the Earth and its biosphere from planetary
     and other extraterrestrial sources of contamination.

2.   SCOPE AND APPLICABILITY

     a.   This Instruction applies to NASA Headquarters and Field
          Installations.

     b.   The provisions of this Instruction cover all space
          flight missions which may intentionally or
          unintentionally carry Earth organisms and organic
          constituents to the planets or other solar system
          bodies; and any mission employing spacecraft which
          are intended to return to Earth and/or its
          biosphere from a planet-target of exploration.

3.   POLICY

     The conduct of scientific investigations of possible
     extraterrestrial life forms, precursors, and remnants must
     not be jeopardized. In addition, the Earth must be
     protected from the potential hazard posed by
     extraterrestrial matter carried by a spacecraft returning
     from another planet. Therefore, for certain space-
     mission/target-planet combinations, controls on organic and
     biological contamination carried by spacecraft shall be
     imposed, in accordance with issuances implementing this
     policy.

*4. MISSION CONSTRAINTS

     Specific constraints imposed on spacecraft involved in solar
     system exploration will depend on the nature of the mission

*Changed by this revision

and the identity of the target body or bodies. These
constraints will take into account current scientific
knowledge about the target bodies through recommendations
from both internal and external advisory groups, but most
notably from the Space Studies Board of the National Academy
of Sciences. The most likely constraints on missions of
concern will be a requirement to reduce the biological
contamination of the spacecraft, coupled with constraints on
constituents of the spacecraft and organic samples, and
restrictions on the handling and methods by which
extraterrestrial samples are returned to Earth. In the
majority of missions, there will also be a requirement to
document spacecraft flyby operations, impact potential, and
the location of landings or impact points of spacecraft on
planetary surfaces or other bodies. The nature and
applicability of mission constraints required by this policy
will be promulgated in subordinate NASA Management
Instructions.

5.   RESPONSIBILITIES

   a.   The Associate Administrator for Space Science and
        Applications is responsible for overall administration
        of NASA planetary protection policy. The management of
        the policy is delegated to the Planetary Protection
        Officer in the Life Sciences Division, who is
        responsible for:

        (1) Prescribing regulations, standards, procedures,
             and guidelines applicable to all NASA
             organizations, programs, and activities to achieve
             the policy objectives prescribed in this
             Instruction.

        (2) Certifying to the Associate Administrator for
             Space Science and Applications and the
             Administrator: prior to launch, and in the case of
             returning spacecraft, prior to the return phase of
             the mission, prior to Earth entry, and again prior
             to approved release of returned materials, that:

             (i)   all measures have been taken to assure
                   meeting NASA policy objectives as
                   established in this Instruction and all
                   lower-tier implementing directives;

        (ii) the recommendations of the applicable
             regulatory agencies with respect to
             planetary protection have been considered
             and their statutory requirements have been
             fulfilled; and

        (iii) the international obligations assessed by
             the General Counsel and the International
             Relations Division have been met, and
             international implications have been
             considered.

    (3) Conducting reviews, inspections, and evaluations
        of plans, facilities, equipment, personnel,
        procedures, and practices of NASA organizational
        elements and NASA contractors to discharge the
        requirements of this Instruction.

    (4) Taking actions as necessary to achieve conformance
        with applicable policies, regulations, and
        procedures.

*b.   The Associate Administrator for Space Flight and the
     Associate Administrator for Space Operations will
     ensure that applicable standards and procedures
     established under this policy are incorporated into
     manned space flight missions to the maximum extent
     possible. Any exceptions will be requested and
     justified to the Administrator through the Office of
     Space Science and Applications.

c.   Program Directors, through the Directors of Field
     Installations, are responsible for:

    (1) Meeting the biological and organic contamination
        control requirements of this Instruction and other
        applicable policies, regulations, and procedures
        during the conduct of research, development, test,
        and preflight operational activities; and

    (2) Providing for the conduct of reviews, inspections,
        and evaluations by the Office of Space Science and
        Applications pursuant to this Instruction.

*Changed by this revision.

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6.   <u>IMPLEMENTATION</u>

      The Associate Administrator for Space Science and
      Applications will assure implementation of this Instruction
      by:

      a.   Maintaining the required activities in support of the
            planetary protection policy at NASA Headquarters.

      b.   Assuring that the research and technology required to
            implement the planetary protection policy is conducted.

      c.   Monitoring space flight missions as necessary to meet
            the requirements for certification.

7.   <u>CANCELLATION</u>

      NMI 8020.7A dated May 4, 1988.

                                          Administrator

<u>DISTRIBUTION:</u>
SDL 1

HISTORY AND BRIEF DESCRIPTION OF THE VIKING CLEANING AND STERILIZING PROCEDURES

Throughout this document, the task group has referred to Viking levels of cleanliness and sterilization as representing a reasonable standard for future Mars missions. It has distinguished between the preparations that may be needed for unpiloted missions that carry no in situ, extant life-detection experiments, and those that will follow later and will carry such life-detection experiments. For the former, the task group has proposed that Viking-level cleanliness will be sufficient, with the caveat that more extensive bioburden assays should be included, incorporating modern techniques as they are adapted for the assay of spacecraft. For missions that include extant life-detection experiments, the task group has recommended use of the Viking sterilization program, or one equally effective in removing bioburden and contaminants. Briefly described below are the very detailed, comprehensive protocols used on Viking, along with some of the scientific and historical rationale underlying these procedures.

The Viking mission was conceived and designed in general in the late 1960s and launched in 1975. It successfully landed two fairly sophisticated spacecraft on Mars (with an orbiter for each), both of which performed almost perfectly and produced an enormous amount of data, far beyond that specified, over a period of up to 3 years, depending on the experiment.

The spacecraft, which were identical, emphasized the search for life on Mars and contained three different experiments, each designed specifically to look for evidence of indigenous, extant biological activity in the surface material. In addition, there was a pyrolysis gas chromatograph-mass spectrometer (GC-MS) designed to search for organic matter in the samples. The remainder of the payload included instrumentation for atmospheric analyses, collection of data on climatology and seismic activity, and preliminary geochemical analyses; a sample acquisition system; and cameras.

It was clear that the life-detection experiments and the GC-MS were all extremely susceptible to contamination (both biological and chemical) from terrestrial sources and **absolutely needed to be protected** from contaminants that would yield false-positive data. It seemed pointless to go to Mars to detect terrestrial bacteria or terrestrial hydrocarbons that were carried there on a contaminated spacecraft. This problem was recognized in the early stages of planning for lunar and planetary exploration, and a planetary quarantine office was established at NASA Headquarters in the early 1960s. This operation included a planetary quarantine officer (a career Public Health officer) and an operating budget for the development of a research program aimed at establishing the necessary technology to prevent planetary contamination. Help was solicited from a wide spectrum of expertise in the country such as the U.S. Public Health Service, the Center for Disease

Control, the Department of Agriculture, the food canning industry, and others, and an interagency committee was established to oversee NASA's activities. At the same time the cooperation of the international community was solicited through the Committee on Space Research (COSPAR), and an international agreement to prevent contamination was produced. The former Soviets are party to this agreement, and although we have never seen their protocols for spacecraft cleaning and sterilization, their officials have assured us that their Mars probes were sterilized.

During the 1960s, a great deal of research was done in universities and in government laboratories to find methods for cleaning and, if necessary, sterilizing spacecraft. Research was done in the area of survivability of microorganisms under extremely adverse conditions, including simulated martian environments. Expert advisory committees were assembled, and guidelines were developed that led to the formulation of the Viking cleaning and sterilization protocols. Many techniques were studied, evaluated, and rejected or accepted. These included most of the contemporary methods for sterilizing found in the biology laboratory, in hospitals, and in the food (particularly canning) industry, as well as techniques for the control of disease. Since large structures, such as spacecraft, had never been sterilized before, many of the seemingly simple questions took on unusual complexities. These problems were compounded by the need to sterilize materials and components that had never undergone such treatment and would very likely be sensitive to it. This necessitated an extensive heat-testing program and the replacement of some standard spacecraft parts with new materials. Although this was a costly undertaking, there is reason to believe that it led to a family of new and more reliable materials for the spacecraft industry.

Sterilization techniques such as gaseous sterilization with "fumigants" such as ethylene oxide were rejected because of the corrosive and potentially explosive nature of the gases. Spacecraft irradiation was rejected because of the sensitivity of many spacecraft components and scientific instruments to irradiation, and because of the great difficulty in implementing such a technique. Furthermore, it had been determined that surface sterilization was inadequate because viable organisms were found in the interiors of components and materials (including plastics) and in cracks and crevices not reached by gases. It was reasoned that such organisms could be released by the impact of landing, thus providing a source of contamination for the surface to be sampled. The biology instrumentation was the system most sensitive to contamination and was the driver in the development of sterilization techniques. Surface samples were to be acquired by a sampling arm, transported to a sample-processing system where they could be sifted and sized, and then moved into whatever instrument was scheduled to receive them. These instruments included the three life-detection instruments, the GC-MS, and the inorganic analytical instrument. Since

there was no realistic way to totally isolate the life-detection instruments, there was no way to sterilize only those instruments, and so the entire spacecraft had to be sterilized.

Heat sterilization proved to be the most satisfactory method available for use. Two methods were considered: wet heat (autoclaving) and dry heat. Since wet heat was too destructive (particularly for electronic components), dry heat was ultimately used. Briefly, after assembly and testing, the Viking spacecraft was disassembled and treated as follows:

1. Surfaces were rigorously cleaned to reduce the starting bioburden on the spacecraft, thus reducing the time required for sterilization at high temperature.

2. Instruments were cleaned and assembled in cleanrooms by workers in surgical attire; laminar flow hoods were used, and the rooms had appropriate filters to remove virtually all microorganisms from the air. Both the lander and orbiter were treated in this way.

3. The entire lander and its payload were assembled under the same conditions. The lander was packaged inside a sealed "bioshield" that prevented recontamination between the period of time from assembly and subsequent sterilization, through launch, until departure from Earth's atmosphere.

4. The lander was then placed in an oven and subjected to dry heat in cycles. In order to assure that the interior of the spacecraft reached sufficient temperature for sterilization, a liquid was pumped to the interior. The precise heating cycle was indicated by the calculated bioburden on the lander as determined by surface sampling with standard laboratory techniques (cotton swabbing, culturing, and microbial colony counting). The temperatures used and the duration of the heat application were calculated to be sufficient to sterilize the entire lander and all of its parts.

The success of this rather cumbersome procedure is found in the success of the mission: no instruments failed. The lander worked perfectly, and no problems were induced by sterilization. The biology instruments and the GC-MS were not contaminated in any detectable way. Although the procedure certainly had an impact on the cost of the mission (it has been estimated at between 5 and 15 percent of the total cost), perhaps the financial aspect was not too serious considering that the scientific potential was preserved. In addition, a catalog of highly resistant and reliable materials and components was produced, which should greatly simplify dealing with the problem of cleaning spacecraft for future missions, as well as substantially reduce future costs.

It is likely that other existing techniques appropriate to future missions may be simpler to implement and more effective. Viking sterilization protocols were settled on in the early 1970s and based on technology devel-

oped in the 1960s and earlier. An appropriate, properly funded research program could greatly enhance the prospect of simplifying procedures and reducing costs. This task group urges NASA to proceed with such a program.

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