Assessment of Planetary Protection Requirements for Spacecraft Missions to Icy Solar System Bodies

Committee on Planetary Protection Standards for Icy Bodies in the Outer Solar System
Space Studies Board
Division on Engineering and Physical Sciences
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Preface

In a letter sent to the National Research Council's (NRC's) Space Studies Board (SSB) Chair Charles F. Kennel on May 20, 2010, Edward J. Weiler, NASA's associate administrator for the Science Mission Directorate (SMD), explained that understanding of the planetary protection requirements for spacecraft missions to Europa and the other icy bodies of the outer solar system should keep pace with our increasing knowledge of these unique planetary environments. Specific advice regarding planetary protection requirements for Europa is contained in the 2000 NRC report *Preventing the Forward Contamination of Europa.*[1] NRC advice concerning other icy bodies is either nonexistent or contained in reports that are now outdated. As NASA and other space agencies prepare for future missions to the icy bodies of the outer solar system, it is appropriate to review the findings of the 2000 *Europa* report and to update and extend its recommendations to cover the entire range of icy bodies—i.e., asteroids, satellites, Kuiper belt objects, and comets. These considerations led Dr. Weiler to request that the NRC revisit the planetary protection requirements for missions to icy solar system bodies in light of current scientific understanding and ongoing improvements in mission-enabling technologies. In particular, the NRC was asked to consider the following subjects and make recommendations:

- The possible factors that usefully could be included in a Coleman-Sagan formulation describing the probability that various types of missions might contaminate with Earth life any liquid water, either naturally occurring or induced by human activities, on or within specific target icy bodies or classes of objects;
- The range of values that can be estimated for the above factors based on current knowledge, as well as an assessment of conservative values for other specific factors that might be provided to missions targeting individual bodies or classes of objects; and
- Scientific investigations that could reduce the uncertainty in the above estimates and assessments, as well as technology developments that would facilitate implementation of planetary protection requirements and/or reduce the overall probability of contamination.

In response to this request, the Committee on Planetary Protection Standards for Icy Bodies in the Outer Solar System was established in September 2010. The committee held organizational teleconferences on November 17 and December 15 in 2010. The committee's first meeting to hear presentations relating to its task took place at the National Academies' Keck Center in Washington, D.C., on January 31 through February 2, 2011. Additional presentations and discussions were heard during a meeting held at the Arnold and Mabel Beckman Center of the National Academies in Irvine, California, on March 16-18 and during a teleconference held on May 13. The committee's final meeting was held at the Beckman Center on June 14-16.

The work of the committee was made easier thanks to the important help, advice, and comments provided by numerous individuals from a variety of public and private organizations. These include the following: Doug Bernard (Jet Propulsion Laboratory), Brent Christner (Louisiana State University), Benton C. Clark (Space Science Institute), Karla B. Clark (Jet Propulsion Laboratory), Catharine A.

[1] National Research Council, *Preventing the Forward Contamination of Europa*, National Academy Press, Washington, D.C., 2000.

Conley (NASA, Headquarters), Steven D'Hondt (University of Rhode Island), Will Grundy (Lowell Observatory), Torrence V. Johnson (Jet Propulsion Laboratory), Ralph D. Lorenz (John Hopkins University, Applied Physics Laboratory), Wayne L. Nicholson (University of Florida), Curt Niebur (NASA, Headquarters), Robert T. Pappalardo (Jet Propulsion Laboratory), Chris Paranicas (John Hopkins University, Applied Physics Laboratory), P. Buford Price, Jr. (University of California, Berkeley), Louise Prockter (John Hopkins University, Applied Physics Laboratory), John D. Rummel (East Carolina University), Daniel F. Smith (Advanced Sterilization Products), J. Andrew Spry (Jet Propulsion Laboratory), John Spencer (Southwest Research Institute), Elizabeth Turtle (John Hopkins University, Applied Physics Laboratory), Christopher R. Webster (Jet Propulsion Laboratory), and Yuri Wolf (National Institutes of Health).

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

The committee wishes to thank the following individuals for their participation in the review of this report: John R. Battista, Louisiana State University; Chris F. Chyba, Princeton University; Gerald W. Elverum, TRW Space Science and Defense; Kevin P. Hand, NASA Jet Propulsion Laboratory; Margaret G. Kivelson, University of California, Los Angeles; Christopher P. McKay, NASA Ames Research Center; Ronald F. Probstein, Massachusetts Institute of Technology; John D. Rummel, East Carolina University; and Yuri I. Wolf, National Library of Medicine, National Institutes of Health.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Larry W. Esposito, University of Colorado, Boulder. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Summary

NASA's exploration of planets and satellites over the past 50 years has led to the discovery of water ice throughout the solar system and prospects for large liquid water reservoirs beneath the frozen shells of icy bodies in the outer solar system. These putative subsurface oceans could provide an environment for prebiotic chemistry or a habitat for indigenous life. During the coming decades, NASA and other space agencies will send flybys, orbiters, subsurface probes, and, possibly, landers to these distant worlds in order to explore their geologic and chemical context and the possibility of extraterrestrial life. Because of their potential to harbor alien life, NASA will select missions that target the most habitable outer solar system objects. This strategy poses formidable challenges for mission planners who must balance the opportunity for exploration with the risk of contamination by terrestrial microbes that could confuse the interpretation of data from experiments concerned with the origins of life beyond Earth or the processes of chemical evolution. To protect the integrity of mission science and maintain compliance with the mandate of the 1967 Outer Space Treaty to "pursue studies of outer space, including the Moon and other celestial bodies . . . so as to avoid their harmful contamination,"[1] NASA adheres to planetary protection guidelines that reflect the most current experimental and observational data from the planetary science and microbiology communities.

The 2000 National Research Council (NRC) report *Preventing the Forward Contamination of Europa*[2] recommended that spacecraft missions to Europa must have their bioload reduced by such an amount that the probability of contaminating a Europan ocean with a single viable terrestrial organism at any time in the future should be less than 10^{-4} per mission.[3] This criterion was adopted for consistency with prior recommendations by the Committee on Space Research (COSPAR) of the International Council for Science for "any spacecraft intended for planetary landing or atmospheric penetration."[4] COSPAR, the de facto adjudicator of planetary protection regulations, adopted the criterion for Europa, and subsequent COSPAR-sponsored workshops extended the 10^{-4} criterion to other icy bodies of the outer solar system.[5,6]

In practice, the establishment of a valid forward-contamination-risk goal as a mission requirement implies the use of some method—either a test or analysis—to verify that the mission can achieve the stated goal. The 2000 *Europa* report recommended that compliance with the 10^{-4}criterion be determined by a so-called Coleman-Sagan calculation.[7,8,9] This methodology estimates the probability of forward contamination by multiplying the initial bioload on the spacecraft by a series of bioload-reduction factors associated with spacecraft cleaning, exposure to the space environment, and the likelihood of encountering a habitable environment. If the risk of contamination falls below 10^{-4}, the mission complies with COSPAR planetary protection requirements and can go forward. If the risk exceeds this threshold, mission planners must implement additional mitigation procedures to reach that goal or must reformulate the mission plans.

The charge for the Committee on Planetary Protection Standards for Icy Bodies in the Outer Solar System called for it to revisit the 2000 *Europa* report in light of recent advances in planetary and life sciences and examine the recommendations resulting from two recent COSPAR workshops. The committee addressed three specific tasks to assess the risk of contamination of icy bodies in the solar system.

The first task concerned the possible factors that could usefully be included in a Coleman-Sagan formulation of contamination risk. The committee does not support continued reliance on the Coleman-Sagan formulation to estimate the probability of contaminating outer solar system icy bodies. This calculation includes multiple factors of uncertain magnitude that often lack statistical independence.

Planetary protection decisions should not rely on the multiplication of probability factors to estimate the likelihood of contaminating solar system bodies with terrestrial organisms unless it can be unequivocally demonstrated that the factors are completely independent and their values and statistical variation are known.

The second task given to the committee concerned the range of values that can be estimated for the terms appearing in the Coleman-Sagan equation based on current knowledge, as well as an assessment of conservative values for other specific factors that might be provided to the implementers of missions targeting individual bodies or classes of objects. The committee replaces the Coleman-Sagan formulation with a series of binary (i.e., yes/no) decisions that consider one factor at a time to determine the necessary level of planetary protection. The committee proposes the use of a decision-point framework that allows mission planners to address seven hierarchically organized, independent decision points that reflect the geologic and environmental conditions on the target body in the context of the metabolic and physiological diversity of terrestrial microorganisms. These decision points include the following:

1. *Liquid water*—Do current data indicate that the destination lacks liquid water essential for terrestrial life?
2. *Key elements*—Do current data indicate that the destination lacks any of the key elements (i.e., carbon, hydrogen, nitrogen, phosphorus, sulfur, potassium, magnesium, calcium, oxygen, and iron) required for terrestrial life?
3. *Physical conditions*—Do current data indicate that the physical properties of the target body are incompatible with known extreme conditions for terrestrial life?
4. *Chemical energy*—Do current data indicate that the environment lacks an accessible source of chemical energy?
5. *Contacting habitable environments*—Do current data indicate that the probability of the spacecraft contacting a habitable environment within 1,000 years is less than 10^{-4}?
6. *Complex nutrients*—Do current data indicate that the lack of complex and heterogeneous organic nutrients in aqueous environments will prevent the survival of irradiated and desiccated microbes?
7. *Minimal planetary protection*—Do current data indicate that heat treatment of the spacecraft at 60°C for 5 hours will eliminate all physiological groups that can propagate on the target body?

Positive evaluations for any of these criteria would release a mission from further mitigation activities, although all missions to habitable and non-habitable environments should still follow routine cleaning procedures and microbial bioload monitoring. If a mission fails to receive a positive evaluation for at least one of these decision points, the entire spacecraft must be subjected to a terminal dry-heat bioload reduction process (heating at temperatures >110°C for 30 hours) to meet planetary protection guidelines.

Irrespective of whether a mission satisfies one of the seven decision points, the committee recommends the use of molecular-based methods to inventory bioloads, including both living and dead taxa, for spacecraft that might contact a habitable environment. Given current knowledge of icy bodies, three bodies present special concerns for planetary protection: Europa, Jupiter's third largest satellite; Enceladus, a medium-size satellite of Saturn; and Triton, Neptune's largest satellite. Missions to other icy bodies present minimal concern for planetary protection.

The advantage of the decision framework over the Coleman-Sagan approach lies in its simplicity and in its abandoning of the multiplication of non-independent bioload reduction factors of uncertain magnitude. At the same time, the framework provides a platform for incorporating new observational data from planetary exploration missions and the latest information about microbial physiology and metabolism, particularly for obligate and facultative psychrophiles (i.e., cold-loving and cold-tolerant microbes).

The committee's third task concerned the identification of scientific investigations that could reduce the uncertainty in the above estimates and assessments, as well as technology developments that would facilitate implementation of planetary protection requirements and/or reduce the overall probability of contamination. The committee recognizes the requirement to further improve knowledge about many of the parameters embodied within the decision framework. Areas of particular concern for which the committee recommends research include the following:

- Determination of the time period of heating to temperatures between 40°C and 80°C required to inactivate spores from psychrophilic and facultative psychrophilic bacteria isolated from high-latitude soil and cryopeg samples, as well as from facultative psychrophiles isolated from temperate soils, spacecraft assembly sites, and the spacecraft itself.
- Studies to better understand the environmental conditions that initiate spore formation and spore germination in psychrophilic and facultative psychrophilic bacteria so that these conditions/requirements can be compared with the characteristics of target icy bodies.
- Searches to discover unknown types of psychrophilic spore-formers and to assess if any of them have tolerances different from those of known types.
- Characterization of the protected microenvironments within spacecraft and assessment of their microbial ecology.
- Determination of the extent to which biofilms might increase microbial resistance to heat treatment and other environmental extremes encountered on journeys to icy bodies.
- Determination of the concentrations of key elements or compounds containing biologically important elements on icy bodies in the outer solar system through observational technologies and constraints placed on the range of trace element availability through theoretical modeling and laboratory analog studies.
- Understanding of global chemical cycles within icy bodies and the geologic processes occurring on these bodies that promote or inhibit surface-subsurface exchange of material.
- Development of technologies that can directly detect and enumerate viable microorganisms on spacecraft surfaces.

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4. The recommendation to accept the 10^{-4} criterion was made at the 7th COSPAR meeting in May 1964 (see COSPAR, *Report of the Seventh COSPAR Meeting, Florence Italy*, COSPAR, Paris, 1964, p. 127, and, also, *COSPAR Information Bulletin*, No. 20, November, 1964, p. 25). The historical literature does not record the rationale for COSPAR's adoption of this standard. Subsequent policy changes restricted the 10^{-4} standard to Mars missions (COSPAR, "COSPAR Planetary Protection Policy (20 October 2002; As Amended to 24 March 2011)," COSPAR, Paris, p. A1, available at http://cosparhq.cnes.fr/Scistr/PPPolicy%20(24Mar2011).pdf.

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1
Current Status of Planetary Protection Policies for Icy Bodies

CONTEXT

The most recent decadal survey for planetary science by the National Research Council (NRC), *Visions and Voyages for Planetary Science in the Decade 2013-2022,* identified “Planetary Habitats: Searching for the Requirements for Life” as one of three crosscutting themes in NASA’s solar system exploration strategy.[1] This theme addresses the key question, Are there modern habitats elsewhere in the solar system with necessary conditions, organic matter, water, energy and nutrients to sustain life? From this perspective, the most interesting bodies to explore present the greatest concern for contamination with terrestrial organisms riding on spacecraft.

Life on Earth, and presumably elsewhere in the solar system, depends on the occurrence of liquid water, sources of energy (chemical and solar), and numerous elements including carbon, hydrogen, nitrogen, phosphorus, sulfur, potassium, magnesium, calcium, oxygen, and iron. NASA’s exploration program to the outer planets has provided strong evidence that some of the icy satellites harbor liquid oceans beneath outer shells of ice that may range in thickness from several kilometers to several hundred kilometers. Because of their potential to inform us about life beyond Earth, these intriguing solar system objects have attracted the attention of the astrobiology community and mission planners. Although NASA has not yet established a mission schedule, anticipated flybys and orbiters pose significant challenges to planetary protection efforts that seek to maintain the pristine nature of these bodies for future scientific investigation. If future mission designs were to include landers or penetrators, the increased likelihood of coming into contact with habitable environments might require more stringent planetary protection procedures.

As a signatory to the United Nations Outer Space Treaty, NASA has developed and implemented policies consistent with the treaty’s requirement that “parties to the Treaty shall pursue studies of outer space including the Moon and other celestial bodies, and conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose.”[2] The Committee on Space Research (COSPAR) of the International Council for Science maintains a planetary protection policy representing the international consensus standard for the “appropriate measures” referred to in the treaty’s language.

The avoidance of harmful contamination to planetary environments can, in its broadest interpretation, be motivated by the protection of extraterrestrial life forms and their habitats from adverse changes and/or by the preservation of the scientific integrity of results relating to those selfsame environments. COSPAR and NASA have adopted the latter interpretation. COSPAR’s planetary protection policies are founded on the principal that “the conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized.”[3] The findings and recommendations of the Committee on Planetary Protection Standards for Icy Bodies in the Outer Solar System resulted from the deliberations conducted within a similar motivational framework.

COSPAR’s planetary protection policy categorizes spacecraft missions according to their type (i.e., flyby, orbiter, lander, or sample return) and the degree to which the spacecraft’s destination might inform the processes of chemical evolution and/or the origin of life (Table 1.1). The policy routinely changes in response to inputs from member organizations, including the NRC, which re-evaluate advances in scientific knowledge in both the planetary and the life sciences.

One such input came in 2000 when the NRC issued the report *Preventing the Forward Contamination of Europa*.[4] The authors of that report were unable to agree on a methodology by which COSPAR's existing categorization system could be extended to cover spacecraft missions to Europa.[5] In place of categorization, the report recommended that spacecraft missions to Europa must reduce their bioload by an amount such that the probability of contaminating a putative Europan ocean with a single viable terrestrial organism at any time in the future should not exceed 10^{-4} per mission.

The 10^{-4} criterion proposed by the authors of the NRC's 2000 *Europa* report is rooted in the history of COSPAR planetary protection policy statements and resolutions. Before its revision in 1982, COSPAR's planetary protection policies were based on a quantitative assessment of the likelihood of contaminating planetary bodies of interest. The 10^{-4} contamination criterion can be traced back to a COSPAR resolution promulgated in 1964 concerning "any spacecraft intended for planetary landing or atmospheric penetration." Unfortunately, the historical literature does not record the rationale for COSPAR's adoption of the 10^{-4} standard. Nor, in, fact has the committee been able to come up with its own quantitative rationale for this number. Even though COSPAR has all but eliminated quantitative approaches from its policy statements, the apparently arbitrary 10^{-4} standard continues to guide the implementation of planetary protection regulations, particularly with respect to those pertaining to missions to Mars.[6] The adoption of a particular contamination criterion raises a number of questions. First, was it appropriate for the authors of the 2000 *Europa* report to apply a martian standard to Europa for any other than historical reasons? The current committee argues that since the advertised purpose of planetary protection is to preserve the integrity of scientific studies relevant to the origins of life and the processes of chemical evolution, the contamination standard for a particular object is directly related to the scientific priority given to studies of that object. Recent NRC reports such as *A Science Strategy for the Exploration of Europa*,[7] *New Frontiers in the Solar System: An Integrated Exploration Strategy*,[8] and *Vision and Voyages for Planetary Science in the Decade 2013-2022*[9] have ranked the scientific priority of studies of Mars and Europa as being, if not equal, then a very close one and two. Thus, a contamination standard applicable to one should, to first order, be applicable to the other.

A second question is determination of the standard itself. It should be possible, in principle, to come up with a standard that is simultaneously not arbitrary and still permits exploration. For example, it could be argued that the standard be such that the likelihood of contamination by spacecraft is less than the likelihood of contamination by meteoritic delivery of Earth microbes in impact-launched meteorites (integrated over some time period, say, the interval of anticipated spacecraft launches). But the adoption of such a standard may preclude the exploration of the icy bodies of the outer solar system.[10]

The committee's decision to retain use of the historical 10^{-4} was predicated on two factors. First, planetary protection policies are deliberately conservative and strongly influenced by historical implementation practices. The 10^{-4} standard is conservative, but implementable, as evidenced by the extensive efforts undertaken to ensure that the Viking missions to Mars and the Juno mission to Jupiter were compliant. Second, the committee's charge specifically focuses on the approach taken by the NRC's 2000 *Europa* report committee and subsequent COSPAR actions related to planetary protection measures for the outer solar system. The introduction of a new contamination standard into the deliberations will, in the committee's considered opinion, complicate the resolution of more serious issues arising from the methodology contained in the 2000 *Europa* report.

COSPAR RESPONSE TO NRC RECOMMENDATIONS

In 2009, COSPAR's Panel on Planetary Protection held two workshops to explore how the NRC's Europan criterion and its underlying methodology might extend to other icy bodies of the outer solar system and simultaneously retain consistency with COSPAR's existing categorization scheme.[11,12] These workshops—held on April 15-17 and December 9-10 in Vienna, Austria, and Pasadena, California, respectively—evaluated new scientific evidence and information not available to the authors of the 2000 *Europa* report. The deliberations at the workshops led COSPAR's Panel on Planetary Protection (PPP) to adopt an extended, but simplified version, of the approach previously recommended by the NRC. The key feature of the PPP's proposal was the division of the icy bodies of the outer solar system into three groups:

1. A large group of objects including small icy bodies that were judged to have only a "remote" chance of contamination by spacecraft missions of all types (Table 1.1; see note c for COSPAR's definition of "remote");
2. A group consisting of Ganymede, Titan, Triton, Pluto/Charon, and those Kuiper belt objects with diameters greater than one half that of Pluto that were also thought to pose a "remote" concern for contamination provided that the implementers of a specific spacecraft mission could demonstrate consistency with the 10^{-4} criterion;[13] and
3. A group consisting of Europa and Enceladus that were believed to have a "significant" chance of contamination by spacecraft missions (see Table 1.1; see note d for COSPAR's definition of "significant").

The significant chance of contamination implies that specific measures, including bioburden reduction, need to be implemented for flybys and for orbiter and lander missions to Europa and Enceladus so as to reduce the probability of inadvertent contamination of bodies of water beneath the surfaces of these objects to less than 1×10^{-4} per mission. In March 2011 COSPAR officially adopted the proposed revisions to planetary protection policy advocated by the PPP.

Based on the findings of the 2009 workshops and the growing scientific data supporting exploratory missions for extant life or clues to the origin and evolution of life on outer planets and icy bodies, NASA asked the NRC (Appendix A) to revisit the conclusions contained in the 2000 *Europa* report and to review, update, and extend its recommendations to cover the entire range of icy bodies—i.e., asteroids, satellites, Kuiper belt objects, and comets.

IMPLEMENTING PLANETARY PROTECTION POLICIES

At one time, COSPAR defined the time period for planetary protection to coincide with the so-called period of biological exploration or, simply, the period of exploration.[14,15] This period refers to the time necessary for robotic missions to determine whether biological systems occur on a potentially habitable planetary body. The committee recognizes that some in the scientific community would support longer periods of planetary protection, perhaps bordering on perpetuity. Indeed, the authors of the 2000 *Europa* report explicitly made this assumption.[16] However, the committee adopts the position that an indefinite time horizon for planetary protection will lead to ad hoc practical solutions that may differ for each mission. The concept of a period of exploration lives on in COSPAR policy, explicitly, only in a single section entitled "Numerical Implementation Guidelines for Forward Contamination Calculations" of an appendix on implementation guidelines.[17] In this context, "the period of exploration can be assumed to be no less than 50 years after a Category III or IV mission arrives at its protected target."[18] However, the first planetary space probes were launched almost 50 years ago, and the exploration of the solar system is still in its infancy. Clearly 100 years is too short, given the multi-decade pace of outer planet missions. Yet the pace of technological change and the length of human civilizations do not provide a sound justification for a period of planetary protection of 10,000 years or more. It is not possible to know with certainty the timeframe of exploration of the solar system, and therefore the committee assumes arbitrarily that it will extend for the next millennium.

TABLE 1.1 COSPAR Planetary Protection Categories

	Category I	Category II	Category III	Category IV
Type of mission	Any but Earth return	Any but Earth return	No direct contact (flyby, some orbiters[a])	Direct contact (lander, probe, some orbiters[a])
Target body[b]	Not of direct interest for understanding of chemical evolution or the origin of life; Group 1	Of significant interest relative to chemical evolution and the origin of life, but where there is only a remote[c] chance of contamination; Group 2	Of interest relative to chemical evolution and the origin of life, but where there is a significant[d] chance of contamination; Group 3	Of interest relative to chemical evolution and the origin of life, but where there is a significant[d] chance of contamination; Group 4
Degree of concern	None	Record of planned impact probability and contamination control measures	Limit on impact probability; passive bioburden control	Limit on non-nominal impact probability; active bioburden control
Planetary protection policy requirements	None	Documentation: planetary protection plan, pre-launch report, post-launch report, post-encounter report, end-of-mission report	Documentation: Category II plus: contamination control, organics inventory (as necessary)	Documentation: Category III plus: probability of contamination analysis plan, microbial reduction plan, microbial assay plan, organics inventory
			Implementing procedures such as: trajectory biasing, cleanroom, bioburden reduction (as necessary)	Implementing procedures such as: partial sterilization of contacting hardware (as necessary), bioshield, monitoring of bioburden via bioassay

NOTE: Category V—all Earth-return missions—has not been included because they are not relevant to this study.

[a]The lifetime of a Mars orbiter must be such that it remains in orbit for a period in excess of 20 years or 50 years from launch with a probability of impact of 0.01 or 0.05, respectively.

[b]*Target body* (Icy bodies mentioned in this report are in boldface):

Group 1: Flyby, Orbiter, Lander: Undifferentiated, metamorphosed asteroids; Io; others to be determined.

Group 2: Flyby, Orbiter, Lander: Venus; Moon (with organic inventory); **Comets**; carbonaceous chondrite asteroids; Jupiter; Saturn; Uranus; Neptune; **Ganymede***; **Callisto**; **Titan***; **Triton***; **Pluto/Charon***; Ceres; **Large Kuiper belt objects (more than half the size of Pluto)***; **other Kuiper belt objects**; others to be determined.

Group 3: Flyby, Orbiters: Mars; **Europa**; **Enceladus**; others TBD

Group 4: Lander Missions: Mars; **Europa**; **Enceladus**; others TBD

*The mission-specific assignment of these bodies to Category II must be supported by an analysis of the "remote" potential for contamination of the liquid-water environments that may exist beneath their surfaces (a probability of introducing a single viable terrestrial organism of $< 1 \times 10^{-4}$), addressing both the existence of such environments and the prospects of accessing them. The probability target of 10^{-4} was originally proposed on the basis of historical precedents in the 2000 NRC report *Preventing the Forward Contamination of Europa*. NASA's formal planetary protection policy has adopted this value as defined in NASA Procedural Requirements (NPR) document 8020.12C. COSPAR has discussed 10^{-4} as the acceptable risk for contamination and formally adopted this value in March 2011 for missions to icy bodies in the outer solar system

[c] In COSPAR usage, the term "remote" specifically implies the absence of environments where terrestrial organisms could survive and replicate, or that there is a very low likelihood of transfer to environments where terrestrial organisms could survive and replicate.

[d] In COSPAR usage, the term "significant" specifically implies the presence of environments where terrestrial organisms could survive and replicate, and some likelihood of transfer to those places by a plausible mechanism.

It is worth noting that the values assigned to the period of exploration and the contamination standard are related. The former allows an upper limit to be placed on the acceptable per-mission likelihood of contamination. In other words, the product of the number of spacecraft missions to a particular body during the period of exploration and the contamination standard must be less than one. Thus, the values of 1,000 years and 10^{-4} are self consistent if no more than one mission is dispatched per decade to each icy body of concern.[19]

The approach adopted by COSPAR for assessing compliance with its 10^{-4} standard for missions to Europa and Enceladus (and to a lesser degree for missions to Ganymede, Titan, Triton, Pluto-Charon, and large Kuiper belt objects) makes use of a methodology—the so-called Coleman-Sagan approach (see Chapter 2)[20,2122]—that involves the multiplication of conservatively estimated, but poorly known, parameters. In the case of Europa, the following factors, at a minimum, appear in the calculation:[23]

- Bioburden at launch;
- Cruise survival for contaminating organisms;
- Organism survival in the radiation environment adjacent to Europa;
- Probability of landing on Europa;
- The mechanisms and timescales of transport to the europan subsurface; and
- Organism survival and proliferation before, during, and after subsurface transfer.

It is notable that COSPAR's approach leaves open the possibility of including additional parameters in the calculation. Indeed, the Juno mission to Jupiter was determined to be compliant with the 10^{-4} standard only after the inclusion of an additional parameter related to the probability that organisms on the Juno spacecraft would survive a high-velocity impact with Europa. The impact-survival parameter was determined via modeling and numerical simulations.

If COSPAR's requirement cannot be met, the spacecraft must be subject to rigorous cleaning and microbial reduction processes until it reaches a terminal, or Viking-level, bioload specification. As its name implies, the terminal specification is that to which the Viking Mars orbiter/landers of the 1970s were subjected. This terminal specification was achieved by sealing the Viking spacecraft in a biobarrier and dry heating the entire assembly to a temperature of >111°C for a period of 35 hours.

The long-standing NASA standard assay procedure determines the number of cultivable aerobic bacterial spores that may exist on flight hardware in order to meet a bioburden distribution requirement. The assay technique originally developed for the Viking missions uses a standard culture/pour plate technique to determine the number of spores in any given sample. The spores serve as a "proxy" representation of the total microbial bioburden on the spacecraft.

Over the past decades, research has greatly expanded the understanding and techniques for finding and culturing microbes, providing a greater depth of knowledge about their viability and adaptability within a variety of environments. Surveys of conserved genes from environmental DNA preparations reveal that the sum of all cultivated microorganisms represents <1 percent of naturally occurring microbial diversity.[24] Extrapolation from the observation that 99 percent of all microorganisms in nature do not readily grow under laboratory conditions suggests that the standard NASA spore assay detects only a small fraction of the different kinds of heat-resistant organisms on a spacecraft (see Chapter 2). This inference implies that measurements of initial bioloads and the adequacy of bioload reduction almost certainly will underdetermine the total number of viable microbes on spacecraft by at least two orders of magnitude.

WHY THIS STUDY IS TIMELY

In addition to the recent changes in COSPAR policy for the icy bodies (see above), significant scientific and programmatic changes warrant a reconsideration of the 2000 *Europa* report. The scientific factors include the following:

- *Significant advances in understanding of Europa and the other Galilean satellites*. The 2000 *Europa* report preceded the conclusion of remote-sensing observations of Europa and the other Galilean satellites by the Galileo spacecraft in 2003. On the basis of more extensive analysis of Galileo data and associated theoretical and modeling studies, the planetary science community has a much better understanding of Europa's internal structure and the thickness and dynamics of its ice shell. The same can be said concerning understanding of the two other icy Galilean satellites, Ganymede and Callisto. See Chapter 4.
- *The discovery of Enceladus' polar plumes*. The 2000 *Europa* report was drafted prior to the beginning of intensive in situ and remote-sensing studies of the Saturn system by the Cassini-Hyugens spacecraft in 2004. Prior observations of Enceladus by the Voyager spacecraft in 1980 and 1981 had revealed that this 500-km-diameter satellite possessed an unusually smooth surface and a circumstantial association with Saturn's tenuous E ring. Cassini observations in 2005 revealed plumes of icy material emanating from discrete points along fissures located near to Enceladus' South Pole. The identification of the plumes not only confirmed that this satellite was the source of the material forming the E ring, but also transformed Enceladus into one of the prime locations of astrobiological interest in the solar system. Whereas an ice shell several kilometers to tens of kilometers thick surrounds Europa's ocean, Enceladus' internal water may communicate directly with the satellite's surface. See Chapter 4.
- *New understanding of Titan's complexity*. In situ observations conducted by the Hyugens lander in 2005, augmented by subsequent remote-sensing studies by the Cassini orbiter, have transformed understanding of Titan's complex environment. Discoveries include the presence of the methane analog of Earth's water cycle and the likelihood of an internal water-ammonia ocean. See Chapter 4.
- *The diversity and complexity of Kuiper belt objects*. Although the discovery of more than 100 Kuiper belt objects (KBOs) significantly smaller than Pluto dates back to the 1990s, new observations have detected several KBOs with diameters comparable to or greater than that of Pluto. Moreover, an anomalously large number of KBOs appear to have satellites, which raises the possibility of tidal heating. Neptune's largest satellite Triton is thought to be a captured KBO that has undergone extensive tidal heating. Images of Triton from Voyager 2 revealed geyser-like activity and an extremely young surface, raising the possibility of geologic activity on other tidally heated KBOs. See Chapter 4.
- *Significant advances in microbial ecology and the biology of extremophiles.* Investigations of extremophiles and novel cultivation techniques have improved understanding of the amazing physiological diversity of microbes and their requirements for growth under nominal and extreme environmental conditions. The sequencing of individual microbial genomes and the mixed genomic analysis (metagenomics) of complex microbial communities has demonstrated unanticipated levels of diversity and the evolutionary significance of horizontal transfer of genes between microbes in reshaping their genomes. Microbes take advantage of this versatility to adapt to new environments, but at the same time these studies inform researchers about the limited range of conditions that individual microbial taxa can tolerate. See Chapter 5.

The programmatic factors include the following:

- *The high priority given to missions to Europa and Enceladus in the first and second planetary science decadal surveys.* The NRC released its first planetary science decadal survey 2 years after the completion of the 2000 *Europa* report.[25] The survey's highest-priority non-Mars mission described the Europa Geophysical Explorer, a flagship-class mission that would orbit Europa and determine whether an internal ocean exists. A Europa orbiter retained its position as the highest-priority non-Mars mission in the most recent planetary decadal survey.[26] Moreover, the decade-plus of study and planning behind the current mission concept, the Jupiter Europa Orbiter, has resulted in a mission far more robust and capable than the minimal orbiter NASA considered at the time of the 2000 *Europa* report. See Appendix B.

- *The internationalization of missions to Jupiter's moons*. The days when NASA alone could conceive, plan, and successfully execute missions to Jupiter and beyond have ended. The European Space Agency (ESA), the Japan Aerospace Exploration Agency (JAXA), and the Russian Federal Space Agency have developed plans for future exploration of the Jupiter system. Most attention has focused on the development of a joint NASA-ESA Europa Jupiter System Mission (EJSM). This concept envisages a combination of independent and coordinated studies of Jupiter and its satellites by a NASA-supplied Jupiter Europa Orbiter and an ESA-supplied Jupiter Ganymede Orbiter. Another possible mission would include a JAXA-supplied Jupiter Magnetospheric Orbiter. The international nature of these missions will require agreed upon criteria and procedures for satisfying planetary protection requirements.
- *Planning for future exploration of Titan and Enceladus*. Interest in a follow-on mission to Cassini-Huygens has focused on the development of the NASA-ESA Titan Saturn System Mission. This concept envisages the deployment of two ESA-supplied in situ elements—a lake lander and a hot-air balloon—delivered by a large and complex NASA-supplied orbiter. Studies of Enceladus could occur before or after orbiting Titan. An alternative mission plan describes a stand-alone Enceladus orbiter. See Appendix B.
- *The initiation of the New Frontiers mission line*. The initiation of the New Frontiers line of principal investigator-led, medium-cost missions represents an important legacy of the first planetary science decadal survey. New Frontiers missions selected by NASA that will target the outer solar system include the New Horizons mission to Pluto-Charon and the Juno mission to Jupiter. The latter will invoke a planetary protection plan that relies on the findings and recommendations of the NRC's 2000 *Europa* report. The most recent planetary decadal survey identified several additional New Frontiers candidates relevant to the subject matter of this report.
- *Possibility of Discovery-class missions to outer solar system bodies*. With the exception of New Horizons and Juno, all expeditions to the outer solar system launched to date correspond to flagship-class missions. The complex power and communications systems required for spacecraft that venture beyond the asteroid belt generally exceed the cost caps of principal investigator-led Discovery missions. The need to flight-test the newly developed Advanced Stirling Radioisotope Generator (ASRG) has opened the outer solar system to smaller missions. The most recent competition for Discovery missions allowed for the potential use of two ASRGs at no expense to the principal investigator. One of the three proposals selected for additional study was the Titan Mare Explorer (TIME), a lake lander. The potential selection of TIME and the possibility of future ASRG-powered Discovery missions to destinations in the outer solar system raise important questions. The one most relevant to this study concerns the compatibility between the financial and temporal constraints placed on the development and launch schedule of Discovery missions and the constraints placed by the potential implementation of complex planetary protection measures. See Appendix B.

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2
Binary Decision Trees

Past efforts to meet COSPAR's planetary protection requirements for the outer planets relied on the so-called Coleman-Sagan formula to calculate the probability that a mission would introduce a single viable microorganism capable of growth on or within a mission destination. The formula typically multiplies together estimates for the number of organisms on the spacecraft, the probability of growth on the target body, and a series of bioload reduction factors to determine whether or not estimates of contamination probability fall below 10^{-4}. COSPAR guidelines require that less than 1 in 10,000 missions will deliver a single viable microbe that is able to grow on a solar system destination, i.e., a 10^{-4} probability of contamination per mission flown. Failure to meet this mandated objective could impose requirements for more stringent cleaning or terminal bioload-reduction procedures comparable to that employed by the Viking missions. In extreme cases, satisfying planetary protection requirements might require spacecraft redesign or cancellation of an entire mission.

PROBLEMS WITH COLEMAN-SAGAN CALCULATIONS

The lack of independence for many bioload reduction factors and minimal precision when assigning values for the initial number of microbes within or on the spacecraft compromises the utility of the Coleman-Sagan formulation as a framework for incorporating planetary protection requirements into mission design. The National Research Council's (NRC's) 2000 report *Preventing the Forward Contamination of Europa*[1] illustrates the application while at the same time recognizes shortcomings of the Coleman-Sagan formulation when estimating the risk of forward contamination. To accommodate new knowledge about extremophiles on Earth, the *Europa* report study committee increased the model complexity by using different bioload reduction factors for physiologically distinct classes of microbes including non-specialized microbes, bacterial spores, radiation resistant spores, and highly radiation resistant non-spore-forming microorganisms. The 2000 *Europa* report acknowledged that its improved methodology continued to rely on the uncertain nature of values for nearly every factor in a chain of "uncorrelated" factors: "The values assigned to individual parameters are not definitive…All parameters are assumed to be independent and uncorrelated."[2] From Appendix A of the 2000 *Europa* report, the Coleman-Sagan formula calculates the probability of contamination by each of the four different classes of organisms, each of which represent four different sensitivities to ionizing radiation. Using the formula

$$N_{Xs} = N_{X0} F_1 F_2 F_3 F_4 F_5 F_6 F_7$$

the authors of the 2000 *Europa* report calculated N_{Xs}, or the number of organisms estimated to survive and grow in the target environment summed across each physiological class, where

N_{X0} = Number of viable cells on the spacecraft before launch,
F_1 = Total number of cells relative to cultured cells,
F_2 = Bioburden reduction treatment fraction,
F_3 = Cruise survival fraction,
F_4 = Radiation survival fraction,
F_5 = Probability of landing at an active site,

F_6 = Burial fraction,
F_7 = Probability that an organism survives and proliferates,
  F_{7a} = Survivability of exposure environments,
  F_{7b} = Availability of nutrients,
  F_{7c} = Suitability of energy sources, and
  F_{7d} = Suitability for active growth.

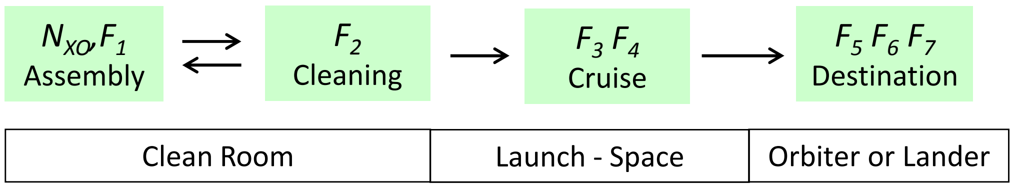

FIGURE 2.1 Mapping the Coleman-Sagan factors to the different phases of a planetary mission. The initial cell counts and cleaning are performed during spacecraft assembly. Survival fraction due to radiation and deep space conditions corresponds to interplanetary cruise; and the characteristics of the planetary destination, either in orbit or within the planetary environment, dictate the remaining factors.

The example calculation in the 2000 *Europa* report shows that the value of N_X (summed across all four physiological classes) had a combined probability of 3.8×10^{-5}; i.e., below COSPAR requirements of 10^{-4}. This approach , which seeks to identify conditions that constrain the sum of N_{Xs} below 10^{-4}, identifies multiple factors that could influence contamination of solar system objects but only if each factor represents an independent process and their values and variances are known.

The committee departs from the conclusions of the 2000 *Europa* report by claiming that not all bioload reduction factors are independent, and with the possible exception of F_5 (probability of landing at an active site) current knowledge makes it impossible to confidently assign values for these factors within orders of magnitude of their true value. Multiplication of uncertain overestimates of bioload reduction factors can lead to unsubstantiated, low estimates of likely contamination. Alternatively, underestimates of bioload reduction coupled with over estimates of bioload on the spacecraft and the flawed assumption that any organism delivered to the target body will grow ($P_g = 1$), would impose unnecessary and possibly unachievable planetary protection demands. The vast majority of different terrestrial microbes have specific requirements for growth that rarely occur in nature or in manipulated laboratory environments. The assumption that $P_g = 1$ in any environment inclusive of icy bodies is conservative. However, the expectation that all microbes can grow anywhere is not supported by available scientific data.

In the example calculation for the NRC's 2000 *Europa* report, the bio-reduction factors F_3 (cruise survival fraction) and F_4 (radiation survival fraction) have a combined bio-load reduction of 10^{-6} to 10^{-11} for the different physiological classes. Yet F_3 and F_4 represent highly correlated, non-independent mechanisms of sensitivity to radiation and vacuum. A significant fraction of the organisms lost due to the combination of ultrahigh vacuum and radiation during the cruise phase will correspond to a subset of those that will succumb during orbit in a high-radiation flux around Europa or other icy moons. The factors F_4 (radiation survival fraction) is part of F_3 (cruise survival fraction), and F_3, F_4, and F_6 (burial fraction) reflect non-independent measures of bio-reduction factor due to radiation flux. In this example, burial fraction dictates the radiation dose profile as a function of depth. The level of protection offered by burial over unit time correlates with estimates of radiation sensitivity as reflected by F_4.

The environmental factors F_{7a} through F_{7d} constrain the survivability of organisms on or in the spacecraft and their ability to proliferate for a combined bio-load reduction of 10^{-6}, but these factors either lack independence or use "survivability" as a substitute for the probability of growth, P_g, which is impossible to estimate. With respect to independence of these factors, F_{7a} will include radiation sensitivity as measured by F_4. The factors F_{7b} through F_{7d} reflect non-independent environmental resources required for growth. The combination of the factors F_{7a} through F_{7d} substitutes for P_g, which most planetary protection studies assume to be unitary because of the complexity of predicting whether a microbe can or cannot grow under a given set of environmental conditions. By assigning probabilities less than 1 for the non-independent bio-reduction factors and the P_g-like estimates for "organism survivability and proliferation," the Coleman-Sagan calculation can reduce the value of N_{Xs} by several orders of magnitude. Yet, with the exception of the geologically influenced parameter F_5, all of these factors have dependencies on other factors.

Even greater uncertainty arises from the inability to confidently assign values to many of these factors, including estimates of the number of viable microbes N_{X0} ,on the spacecraft prior to launch. As described in Chapter 1 of this report, the standard NASA assay of heat-resistant microbes serves as an indicator of the number of spores on the sampled spacecraft surfaces. These measurements provide no information about the number of heat-sensitive but radiation and vacuum resistant microbes on a spacecraft, nor do these surveys provide accurate estimates of heat-resistant spores that are refractory to cultivation. Over the past two decades culture-independent microbial diversity investigations based on comparisons of highly conserved sequences (ribosomal RNA genes) in Bacteria and Archaea demonstrate that microbiologists have successfully cultivated only a small fraction (<1 percent) of the different kinds of single-cell organisms that occur in nature.[3] Deep-sequencing surveys suggest that microbial diversity may be 1,000 to 10,000 times greater than estimates from cultivation-based studies and that most of this novelty corresponds to low abundance taxa described as the "rare biosphere."[4,5] Similar analyses of simple mock communities containing one or a few taxa suggested that sequencing errors can lead to inflated estimates of microbial diversity.[6] More recent studies show that a 2 percent single-linkage preclustering methodology followed by an average-linkage clustering based on pair-wise sequence alignments more accurately predicts expected complexity of mock communities of known taxonomic composition. However, this analytical paradigm does not reduce the fraction of novel taxa in the long-tailed rank abundance curves that define the rare biosphere for complex, naturally occurring microbial communities. This implies that the standard spore assay likely underdetermines the number of heat-resistant organisms on a spacecraft. If many spore-forming organisms cannot grow under laboratory conditions, then growth-based assays of survival will not accurately report the size of the surviving populations.

Because the overall uncertainty factor in the final result from the Coleman-Sagan equation is greater than the uncertainty factor for the least constrained variable, a three or four order of magnitude uncertainty in estimates of the number of organisms on spacecraft would lead to approximately a three or four order of magnitude uncertainty in the overall probability of contamination.

Given current technology, non-rigorous estimates of N_{X0} can lead to significant underestimates of the number of organisms delivered to the target body. Estimates for other bioload reduction factors suffer similar uncertainties. The current inability to cultivate most of the different microbes that comprise a community makes it impossible to estimate what fraction of a community succumbs to radiation and ultralow vacuum during cruise or orbit in a high-radiation environment. Because the overall uncertainty factor in the final result from the Coleman-Sagan equation is greater than the uncertainty factor for the least constrained variable, a 3 or 4 order-of-magnitude uncertainty in estimates of the number of organisms on spacecraft would lead to approximately a 3 or 4 order-of-magnitude uncertainty in the overall probability of contamination.

The most robust estimate for independent factors in the 2000 *Europa* report describe the likelihood that a spacecraft will impact an active area. For example, as described in Chapter 4, calculating the fraction of the surface area that might theoretically communicate with a subsurface ocean over a given period of time yields a first order approximation of the likelihood that microbes on the spacecraft might contaminate the ocean. Under this scenario, the probability of contamination would be estimated according to where and how the spacecraft impacts a surface, rather than deriving uncertain estimates from a series of difficult to determine bio-reduction factors. Multiplying the number of surviving organisms on the spacecraft by the chance that the spacecraft will encounter an area of active surface-subsurface transport, implicitly assumes that each organism or class of organisms has an independent chance of encountering the active area. Yet the probability that two different organisms on the same spacecraft will be transported to the subsurface is tightly correlated; either the spacecraft will land in the active area, in which case most of the spacecraft's surviving bioload can contaminate the subsurface environment, or the spacecraft will land in an inactive area, in which case even a highly contaminated spacecraft cannot affect the subsurface.

COSPAR'S SIMPLIFIED VERSION OF THE COLEMAN-SAGAN APPROACH

As mentioned in the previous chapter, following on the discussions and deliberations at two workshops held in 2009, COSPAR's Panel on Planetary Protection (PPP) ultimately recommended the adoption of a simplified version of the Coleman Sagan approach presented in the NRC's 2000 *Europa* report. Similarly, the simplified recommendations in the formulation described in the COSPAR Planetary Protection Policy, 20 October 2002, as amended and the COSPAR Workshop on Planetary Protection for Outer planet Satellites and Small Solar system Bodies (Vienna Austria 2009) and the COSPAR Workshop on Planetary Protection for Titan and Ganymede (2009) include in its most simplified form:[7]

- Bioburden at launch;
- Cruise survival for contaminating organisms;
- Organism survival in the radiation environment adjacent to Europa;
- Probability of landing on Europa;
- The mechanisms and timescales of transport to the europan subsurface; and
- Organism survival and proliferation before, during, and after subsurface transfer.

However, the same arguments the committee leveled against the more complex approach presented in the NRC's 2000 *Europa* report (see above) apply to simplified formulation adopted as official COSPAR policy. For example, current technology, including the NASA standard spore assay and culture-independent molecular technologies, display a wide variance over many orders of magnitude when estimating bioburden at launch (Bullet 1). Organism survival and cruise survival (bullets 2 and 3) are not independent processes. The timescales of transport to the Europan subsurface (Bullet 5) are also not independent of radiation survival during cruise or in environments adjacent to Europa—they effectively use the same biological information to estimate parameters that affect an organism's ability to survive radiation exposure. Moreover, the policy's open-ended nature—i.e., the possibility of adding additional numerical factors to the calculation (as was done for the Juno mission—potentially compounds issues relating to statistical uncertainty and nonindependence.

Based on these observations and conclusions, the committee saw no scientifically or logically defensible path for improving estimates of factors for the Coleman Sagan formulation as called for in its charge (see Appendix A). In order to make progress, the committee explored the utility of a binary decision matrix similar to that previously employed in the NRC report *Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies: Framework for Decision Making*.[8] Such an approach has already been adopted by COSPAR for determining whether or not sample-return missions from small solar system bodies are classified as restricted or unrestricted Earth-return missions.[9]

AN ALTERNATIVE TO THE COLEMAN-SAGAN FORMULATION

A binary decision-making framework (Figure 2.2) provides an alternative to Coleman-Sagan estimates of contamination that are constrained by uncertain and possibly unknowable factors. The decision framework should consider the habitability of different solar system objects, including environmental conditions necessary for propagation of terrestrial life (see Chapter 3 for details), the probability of transport to a subsurface, habitable environment (see Chapter 4 for details), and the ability of terrestrial organisms to survive nominal bioload reduction treatments and adapt to non-terrestrial environments (see Chapter 5 for details). When the decision framework indicates that contamination would occur if the spacecraft impacted the surface, stricter planetary protection efforts would be required. It should be noted that the binary decision framework presented in Figure 2.2 can be presented in alternative formats, such as an event sequence diagram (Appendix C), which indeed may be preferred in the engineering community.

CONCLUSIONS AND RECOMMENDATIONS

The committee expresses caution about the use of the Coleman-Sagan approach for assessing the risk of forward contamination. The uncertainty in assigned values for initial bioloads and bioload reduction factors, and the multiplication of factors that are not mutually independent, cannot provide robust estimates of the probability of forward contamination.

In contrast, a binary decision-making framework would provide a more robust basis for determining the appropriate level of planetary protection for a given mission, because such a procedure would not compound inaccurate and non-independent estimates of probability factors. Separate and independent decision points in the framework should consider different parameters that define the habitability of the target solar system object(s), the probability of transporting terrestrial organisms to a habitable environment on a given target body, and the ability of terrestrial organisms to endure bioload reduction treatments and subsist in non-terrestrial environments.

> ***Recommendation:*** **Approaches to achieving planetary protection should not rely on the multiplication of bioload estimates and probabilities to calculate the likelihood of contaminating solar system bodies with terrestrial organisms *unless* scientific data unequivocally define the values, statistical variation, and mutual independence of every factor used in the equation.**

> ***Recommendation:*** **Approaches to achieving planetary protection for missions to icy solar system bodies should employ a series of binary decisions that consider one factor at a time to determine the appropriate level of planetary protection procedures to use.**

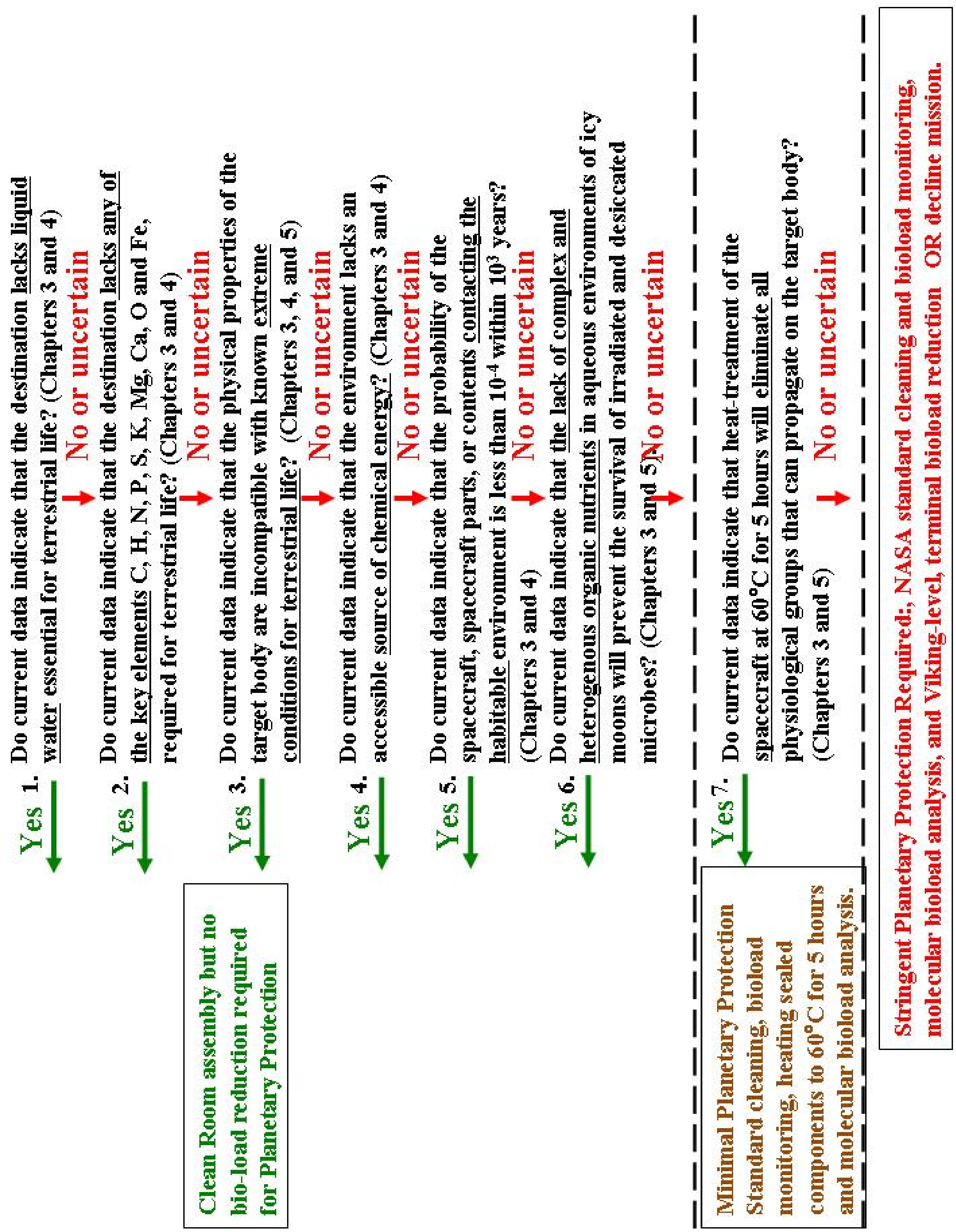

FIGURE 2.2. Binary decision making framework for planetary protection of icy solar system bodies. “Yes” answers to Decision Points 1-6 release the mission from rigorous planetary protection procedures. Whereas a “Yes” to Decision Point 7 requires moderate heating of sealed components. “No” answers to Decision Points 1-7 will require stringent planetary protection procedures, e.g., terminal bioload-reduction or mission cancellation. The phrase, “does current data indicate,” conveys a scientific consensus about the reliability of available information at the time of assessing planetary protection risk.

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3
Hierarchical Decisions for Planetary Protection

Decisions about planetary protection of icy bodies and other solar system destinations must initially assess their habitability by considering environmental conditions that terrestrial microbes can tolerate and by evaluating empirical data for essential elements or other requirements (e.g., water, energy sources). The lack of water on dry rocky moons such as Io would mean that missions to this body would not require planetary protection. On the other hand, if the physical and chemical environment of an icy body might be compatible with growth of terrestrial life, mission planners must assume it to be habitable. Knowledge acquired in areas of biological (primarily microbiological) science over the past 20 years provides important guidance for defining habitability for icy bodies. We can define terrestrial life fairly precisely with regard to its composition and needs for metabolic generation of energy. If the target site does not provide these basic needs, mission planners need not take special precautions normally associated with preventing forward contamination beyond the routine cleaning and monitoring of spacecraft. This approach restricts the number of bodies of concern for planetary protection requirements. Based on current understanding, the outer solar system icy bodies Europa, Enceladus, Titan, and Triton are most relevant to this discussion (see Chapter 4, and see Appendix B for a summary of exploration plans for icy bodies). It should be stressed that designating a body as being habitable does not just refer to the surface of a body, but any microenvironments that might exist within the body (e.g., the subsurface, the atmosphere, etc.). The Decision Points 1-7 given in Figure 2.2 represent hierarchical organization of environmental features that relate to habitability—from the most constraining to the least constraining. For example, since all terrestrial life requires liquid water, the complete absence of water would render all other considerations of habitability irrelevant for planetary protection.

DECISION POINTS

Such considerations as outlined in Chapter 2 and above led the committee to the definition of seven binary decision points. Subsequent subsections will outline each of the decision points. A more detailed discussion of these decision points can be found in Chapters 4 and 5. The answers for different decision points will vary for different objects as will our level of confidence. The framework's language "Do current data indicate..." makes the implicit statement that the preponderance of data supports a particular answer but new information could strengthen or alter the outcome of the decision points.

Decision Point 1—Liquid Water

All life on Earth requires liquid water for protein-based enzymes to function properly. Even for those systems in which extracellular electron transport (EET) to an extracellular substrate occurs,[1,2,3] liquid water remains an absolute requirement. Mission planners should consider any body that lacks liquid water to be non-habitable for terrestrial life.

Decision Point 2—Key Elements

All life on Earth requires carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and a large number of elements in trace concentrations: 70 in all are either required or influence the physiology and growth of various species.[4] Specific transition metals often serve as electron acceptors and donors for catalytic activity or play a role in protein structure. While the literature describes many of the biological functions of trace elements, we have far less information about minimum concentrations of the different trace elements required by organisms and their transport into the cell. In oligiotrophic aquatic environments, iron, molybdenum, and phosphorus limit the extent of primary production and thus other microbial autotrophic and heterotrophic metabolic activity. Because of its importance in all metabolic pathways, phosphate is likely the most important limiting nutrient for marine primary production.[5] If mission planners can confidently demonstrate that the concentration of any one of these elements falls below minimal levels required for microbial growth, the icy body should be considered non-habitable.

Decision Point 3—Physical Conditions

Physical and chemical extremes restrict the distribution of life. Knowledge of how microbes solve the problems of growth in extreme conditions, such as temperature, pH, Eh, and other variables, expands as our study of extreme environments develops. Nevertheless, physical extremes (e.g., temperatures above 122°C,[6] or below −15°C define the known temperature constraints for the replication of terrestrial (carbon-based) life although metabolic activity can occur as low as −20°C.[7] The high temperature range relates to the stability of hydrogen bonds within liquid water at very high temperatures, while the low temperature range relates to the absence of available water molecules in the liquid state. If conditions outside the known limits exist throughout a target body, then it cannot support terrestrial life and should be considered non-habitable.

Radiation also presents a physical challenge to the survival of terrestrial organisms both during flight and on or near the surface of the target icy bodies. Radiation causes DNA double stranded breaks that must be repaired if an organism is to survive. However as discussed below (see Decision Point Six) and in Chapter 5, these repair processes require complex organic compounds.

Decision Point4—Chemical Energy

Life requires a source of chemical or solar energy. Typically electron donors (reductants) coupled with acceptors (oxidants) form electron transport chains that provide chemical energy for living cells. The discovery of microorganisms that use novel redox couples and are capable of surviving chemical and physical extremes previously thought to be inhospitable to life has widened the range of recognized habitable environments.[8] In the terrestrial deep subsurface, some sources of electron donors and acceptors, such as the production of hydrogen by radiolysis of water, show that extreme geophysical environments analogous to those on icy bodies in the outer solar system can be the source of half reactions. In contrast to potential sources of chemical energy, light capture would not provide a useful energy source for terrestrial life forms on the surfaces of, or beneath the thick ice shells of, planetary bodies in the outer solar system. This latter scenario would require would require the unlikely evolution of a photosynthetic apparatus tuned to the spectral qualities of the subsurface photon source. The former scenario contamination is equally unlikely because the liquid water necessary for the survival of photosynthetic life forms would freeze and such organisms would potentially be exposed to unsurvivable radiation fluxes. For these reasons, the subsequent discussion focuses on chemical energy sources.

Although there is no conclusive information available concerning the presence of the chemical energy and elemental sources necessary to support the growth of potential contaminating organisms on icy bodies, the committee assumes they are available. Electron donors (e.g., Fe^{2+}, SH^-, organic carbon) and electron acceptors (e.g., CO_2, SO_4^{2-}, O_2, H_2O_2)[9,10,11,12,13] might be present on some icy bodies. If new data from future missions unequivocally demonstrate the absence of electron donor-receptor pairs on a targeted icy body, then it cannot support terrestrial life and should be considered non-habitable."

Decision Point 5—Contacting Habitable Environments

If a target site cannot be designated as non-habitable by criteria outlined by Decision Points 1-4, then mission planners must consider the probability of the spacecraft coming into contact with potentially habitable regions (see Chapter 4). The decision framework does not differentiate between mission mode, i.e., flybys versus landers versus orbiters in orbits that are either stable or unstable. Instead, Decision Point 5 focuses on the geophysical features of the target body. If the probability of the spacecraft, spacecraft parts, or contents contacting a potentially habitable region as defined by Decision Points 1-4 is less than 10^{-4} within 1,000 years (i.e., over the time period of biological exploration), then no bioload-reduction for planetary protection is required. Each mission must calculate the probability of contacting a habitable environment over the time period of biological exploration, based on the design and architecture of the mission, and based on the geophysical properties of the target body.

Decision Point 6—Complex Nutrients

If nutrient conditions available in liquid environments of an icy body are deemed insufficient to support growth and/or recovery from irradiation and/or desiccation (Chapter 5), then that body cannot support terrestrial life and should be considered non-habitable.

Decision Point 7—Minimal Planetary Protection

If nominal heat treatment (e.g., 60°C for 5 hours) or other bioload-reduction technologies cannot eliminate those physiological types that might have the capacity to grow on the target body (Chapter 5), mission planners must meet NASA's Viking-level, terminal bioload specification (see Chapter 1). Failure to meet this final decision point would require total redesign or cancellation of the mission.

CONCLUSIONS AND RECOMMENDATIONS

A series of decision points based on constraints defined by the preponderance of available scientific data or new information from future missions and research provide a robust mechanism for evaluating planetary protection requirements. The first and most critical decision point must consider whether liquid water is not available, followed by decision points describing the lack of availability of building blocks including the key elements carbon, nitrogen, phosphorus, and so on—the absence of environmental parameters known to be compatible with the growth of terrestrial life—and finally the lack of available energy sources required for terrestrial life. If negative answers to the initial Decision Points 1-4 fail to eliminate a requirement for planetary protection, mission planners must either demonstrate that the probability of a mission coming into contact with a habitable region is less then 10^{-4} over a 1,000-year time frame or that nutrient conditions will not support microorganisms' growth and/or recovery from irradiation and desiccation. Finally, if nominal heat treatment at 60°C for 5 hours will not eliminate microorganisms that are likely to grow on the target body, then Viking-level terminal bioload reduction will be required.

Recommendation: **NASA should adopt a binary hierarchical decision-making framework whereby affirmative answers to any decision point indicating the absence of a factor critical to life as currently known would eliminate further requirements for planetary protection measures.**

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4
A Geophysical Perspective and Inventory of Habitable Environments on Icy Bodies

The geophysical context of solar system objects constrains the potential propagation of terrestrial organisms with known minimal nutritional requirements and environmental tolerances outlined in Chapter 3. The outer solar system contains a broad diversity of icy bodies, ranging from co-accreted satellites bound to their gas giant parent planets to small icy leftovers of planet-like comets, Centaurs (whose orbits cross the giant planets), and the Kuiper belt objects (KBOs). Icy bodies can be divided into categories by size: large icy bodies (radius > 1,000 km, like Europa, Ganymede, Callisto, Titan, Triton, and large KBOs like Pluto and Eris), mid-size icy bodies (200 to 1000 km-radius objects like Mimas, Enceladus, Tethys, Dione, Rhea, Iapetus, Miranda, Ariel, Titania, Umbriel, Oberon, Charon, most known KBOs, and the asteroid Ceres), and small icy bodies that are small enough to avoid becoming spherical (<200 km, like Phoebe, Hyperion, Nereid, comets, Centaurs, and ring moons).

GEOPHYSICAL BOTTLENECKS

The cold and inhospitable surfaces of icy bodies in the outer solar system serve as a natural barrier to forward contamination of their warmer and more hospitable interiors. Here, we describe the geophysical "bottlenecks" that separate terrestrial organisms hitchhiking on a spacecraft from entering potentially habitable environments existing within icy bodies. This chapter first outlines the properties and locations of potentially habitable environments, discussing Decision Points 1 through 4 from Chapter 2. The bulk of the chapter concerns Decision Point 5, discussing transport processes that may operate between the uninhabitable surface and potentially habitable subsurface environments. The chapter concludes with a survey of icy bodies to delineate areas of concern for planetary protection.

The reconnaissance of icy bodies in the outer solar system is incomplete, and in many places basic surface and interior properties remain unknown. We have data from only half of the surfaces of the objects in the Uranus and Neptune systems, and we lack close spacecraft observations for all objects beyond the orbit of Neptune. Interior structures of the satellites of Jupiter and Saturn are constrained by the moment of inertia, which has been measured during close flybys. However, the interpretation of the moment of inertia value in terms of an interior density profile produces results that are not unique.[1] As a result, the reported depths and densities of interior layers are inferences based on assumed common materials that could make up the interior of the body. For other bodies that lack flyby data, interior states represent well-informed guesses. The chemical composition of most bodies is constrained by infrared spectroscopy, which senses only the top few microns of the surface. The only bodies for which deeper knowledge is available are Saturn's moons Enceladus (where active plumes spew water and other materials from its interior);[2] and Titan (where the Huygens probe obtained in situ data about the composition of volatiles in the atmosphere and the upper centimeters of the surface).[3]

POTENTIALLY HABITABLE ENVIRONMENTS

Decision Point 1—Liquid Water

Terrestrial life has a requirement for liquid water. Because water ice serves as the "bedrock" on an icy body, the existence and location of liquid water within the body is key to gauging its habitability. Recent exploration in the outer solar system has revealed that many icy moons have liquid water oceans buried beneath several kilometers or tens of kilometers of ice. Magnetometer data provides compelling evidence of liquid water for Jupiter's moons Europa, Ganymede, and Callisto.[4] Oceans are suspected to be present within Saturn's moons Titan and Enceladus.[5,6] Theoretical considerations of radiogenic heating within large and mid-size icy bodies show that heat dissipation is commonly sufficient to melt ice more than 100 km from the surface.[7] Once melted, internal oceans may also dissipate enough heat to prevent them from freezing.[8] These subsurface oceans are gravitationally and thermodynamically stable over time because liquid water is denser than water ice, the low-density phase present on the surface.

Mechanisms for generating liquid water on an icy body include contact with rocky material warmed by tidal heating, shock heating in a hypervelocity impact, tidal heating within the ice, contact of pure water ice near its melting temperature with contaminated ice mixtures that melt at lower temperatures,[9] and warming of ice by a perennial heat source (e.g., a radioisotope power system) delivered to the target by the spacecraft. Liquid water may exist in intimate association with the ice, for example terrestrial organisms in sea ice can survive below-freezing conditions within microscopic brine pockets at ice grain boundaries.[10] Except for Titan, icy bodies lack a significant atmosphere. On these airless bodies, direct warming of surface ice will lead to sublimation instead of melting, and liquid water that becomes exposed at the surface will not just pool sedately and freeze, but it will undergo rapid freeze-boiling.

Localized melting of ice by a radioisotope power system (RPS) is not likely to present a serious concern for future missions to the outer solar system. Studies were conducted at the Jet Propulsion Laboratory in the late 1990s and early 2000s in support of efforts to design an RPS-powered, ice-penetrating probe for application on a future mission to Mars and Europa.[11] In addition for the need to seal the heat source within Europa's ice so as to raise the vapor pressure to a sufficiently high value to initiate melting, the study revealed the critical power needed if any melting was to take place at all. The study team reported the following: "0.6kW thermal input did not provide enough energy to raise the ice temperature (−170°C) sufficiently to initiate melt. The Europa ice is so cold it acts as an infinite heat sink and the heat is transmitted into the heat sink so quickly that localized phase change at the vehicle shell is impossible. Melt was initiated at 0.8 kW, but with no margin for error on the actual ice temperature. At 1 kW, phase change at the vehicle shell interface was sustainable with the creation of about 1-mm melt-water jacket around the vehicle."[12]

Current outer solar system missions, such as New Horizons mission to Pluto and the Cassini Saturn orbiter, are equipped with the so-called General Purpose Heat Source-Radioisotope Thermoelectric Generators (GPHS-RTG), each of which has a thermal output of 4.5 kW (at the beginning of the mission). So it is conceivable at a single GPHS-RTG could initiate local melting if its plutonium-238 heat sources remained sufficiently intact following impact with an icy body. However, future plans for missions (see Appendix B) to objects of planetary protection concern (e.g., Europa and Enceladus) envisage the use of the Advanced Sterling Radioisotope Generator (ASRG). Each ASRG has a thermal output of only 0.5 kW (at the beginning of the mission). So ASRG's are unlikely to initiate local melting, except in the unlikely case where multiple ASRGs surviving impact while maintaining intimate contact with each other.

In contrast to large or mid-size icy bodies that might contain liquid water in their interior, the nonspherical geometry of small icy bodies indicates that the vast majority of their interiors have remained cold, stiff, and completely solid. Such objects are small enough that they do not contain enough energy (e.g., from radiogenic heating) to generate interior melt during their long-term thermal evolution. Thus small satellites, ring particles, comets, and Centaurs can be eliminated from being bodies of concern for planetary protection.

Decision Point 2—Key Elements

In addition to abundant oxygen and hydrogen on icy surfaces, key biological elements carbon, surlfur, and nitrogen may also occur in some icy surfaces in the form of ice, clathrates, or simple organics. The elements potassium, magnesium, calcium, iron, and phosphorus can dissolve in liquid water that has been in contact with rocky materials. However, in extraterrestrial environments, the bioavailability of compounds containing these elements may limit their use by terrestrial microorganisms. For example, chemical modeling by Pasek and colleagues predicted that phosphine instead of phosphate will account for available phosphorus on Titan.[13] We cannot yet constrain the cycling and bioavailability of different chemical forms of individual elements important to life or their occurrence on icy bodies in our solar system. Knowledge of chemical composition for satellites other than Enceladus and Titan comes mostly from spectroscopy, which only senses the outer few microns of the surface. Volatile frost deposits on the surfaces of icy bodies may not represent their interior chemical composition, making it difficult to assess the abundance of dissolved elements within icy bodies. Therefore, this decision point currently serves a role of intellectual completeness rather than a key hinge point for planetary protection decisions. However, someday this decision point may play a more important role in planetary protection policy in response to new information about the chemistry of icy bodies and minimal element requirements for the propagation of microorganisms.

Decision Point 3—Physical Conditions

The range of possible temperatures of liquid water environments within icy bodies is more tightly constrained than the chemical composition. Reservoirs of liquid water within icy bodies always remain in contact with ice, and thus the temperatures within these liquids hover near the freezing point of pure water (which is a minimum of −20°C at a depth of ~100 km in a large icy body) or mixed ice+salts or ice+ammonia (plausibly as low as −97°C). A source of energy within an ice shell will generally melt the surrounding ice while maintaining the liquid body at the freezing point. In a subsurface ocean overlain by a floating ice shell, the tendency of warm liquid to rise and cool liquid to sink will pin the entire ocean temperature near the freezing point. Heating within such an ocean will cause melting in the overlying ice but will not change the temperature of the water. Under special circumstances, such as a fresh water ocean[14] or if warm saline fluids were injected into the bottom of the ocean,[15] a subsurface ocean may become stratified so that the lower layers of the ocean can warm to above freezing but not above 4°C (or 6°C if adiabatic compression at the bottom of a large icy satellite ocean is taken into account).

The only place where the water temperature may rise above this upper limit lies beneath the base of a subsurface ocean in contact with rocky materials. Cracks within a rocky ocean floor would permit infiltration of water, and contact with warmer rocks at depth can lead to porous convection. Such convection is typified by broad downwellings into the porous rocks balanced by focused upwellings of warm water at hydrothermal vents. The spacing and power output of these hydrothermal systems depends on multiple uncertain assumptions about the nature of the seafloor and the energy source driving the activity.[16,17] The mass flux of fluid transport for a given change in fluid temperature is lower on icy bodies compared to Earth because lower gravity leads to slower convective velocities. Once emitted from the ocean floor, hydrothermal fluids rapidly mix with the surrounding ocean, such that the water temperatures are within a degree of the surrounding ocean within tens of meters from the vent.

Decision Point 4—Chemical Energy

Our knowledge of available redox couples that can provide chemical energy for terrestrial organisms suffers from greater uncertainty than our knowledge of available chemical elements. For icy bodies with liquid water in contact with a rocky interior, water-rock chemical reactions can provide the energy for life. On the largest icy bodies (Ganymede, Callisto, and Titan), ocean water lying between low-pressure ice-I shell above and denser high-pressure ice phases below would not react with the bulk of the rocky interior.[18] Radioactive decay could hydrolyze water on a small scale and provide small amounts of chemical energy.[19] Material transport from the surface of a body to an interior ocean could maintain a chemical energy, for example due to oxidants produced by irradiation of Europa's surface.[20] If appropriate energy sources occur on an icy body, terrestrial biology would only persist if active geochemical cycles occurred between the liquid and the surface or the liquid and the deep interior. As described under Decision Point 2, planetary protection considerations for future missions will have the advantage of research initiatives that provide new information including the availability of biologically relevant sources of energy on icy bodies. In the absence of such information about energy sources and bioavailability of minimal element requirements, it is assumed that any liquid water within poorly characterized icy bodies may have the proper chemistry for supporting terrestrial life.

Decision Point 5—Contacting Habitable Environmemnts

Floating outer ice-I shells may be a frustrating impediment to life-detection experiments, and they serve as a protective barrier from the viewpoint of planetary protection. Therefore, setting planetary protection guidelines requires an understanding of the physical processes that allow vertical transport of material between the subsurface and surface of an icy body and the timescales on which transport occurs. Some of these vertical transport processes operate from the top down, while others operate from the bottom up (Figure 4.1). There are usually limits to the vertical range over which the processes operate; for example, impact gardening and radiation transport material vertically over ~1-m scales, comparable to the physical size of the spacecraft. Cracks open beneath the surface and may penetrate to ~ 1 km depth. Solid-state convection operates within the solid portion of the ice shell, but on most bodies it is confined beneath a stagnant lid that is several kilometers thick. Lithostatic stress limits the propagation depth of cracks in the top of brittle surface materials. Solid-state convection operates within the solid portion of the ice shell but on most bodies, convection is confined beneath a so-called stagnant lid that is several kilometers thick. The stagnant lid is composed of cold material that is so viscous that it cannot participate in convection.[21] If there is no overlap between top-down and bottom-up vertical transport processes, a "no-man's land" exists in the middle of the ice shell that interrupts exchange of material between the surface and a subsurface ocean.

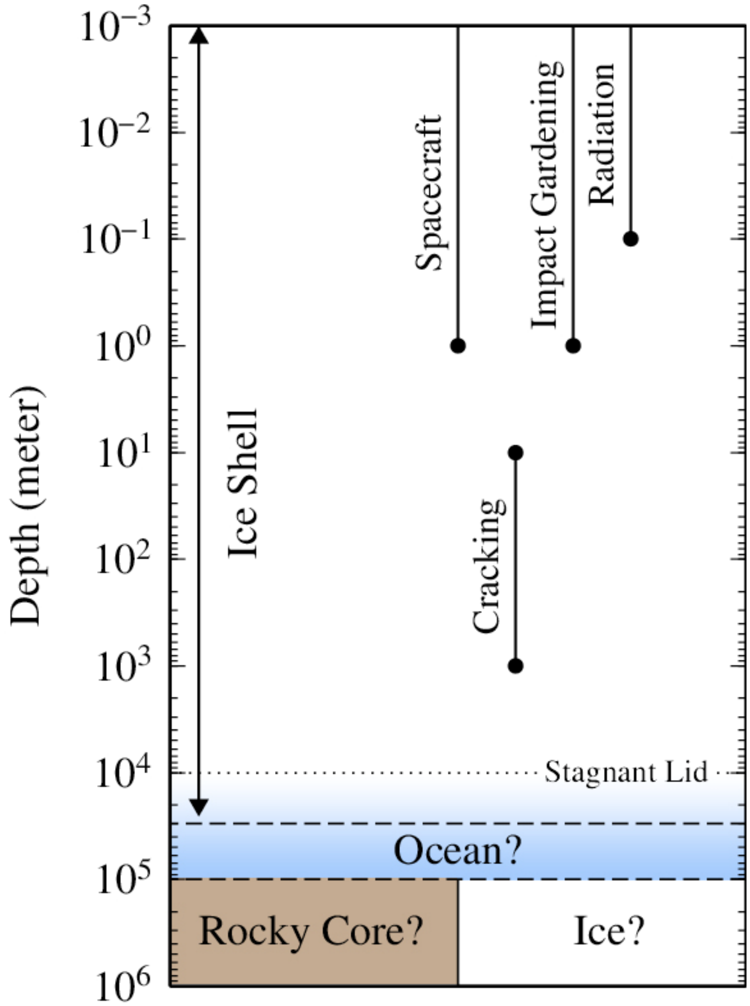

FIGURE. 4.1 Depth of penetration of various vertical transport mechanisms on the surface of a generic icy moon with a ~10- to 100-km-thick ice shell (most applicable to bodies of concern—Europa, Enceladus, Titan, and Triton).

Impact Gardening

At the very top surface of an icy body without an atmosphere, where exogenous contaminants are likely to be deposited, impact gardening dominates the mixing of these materials into the subsurface. Gardening refers to the churning of surface regolith driven by the impact of meteoroids and the subsequent burial of neighboring surface materials by impact ejecta. Phillips and Chyba estimated that on Europa's surface gardening could mix loose surface materials to a depth of ~1 meter over 10 million years,[22] although this burial may be episodic rather than continuous, as about 95 percent of the small craters on Europa may be secondary craters.[23] The impact rate on Europa is within a factor of 2 of the highest impact rates of any icy bodies in the outer solar system because of its location deep inside the Jovian gravity well.[24] Therefore the other icy bodies, with similar to or lower impact rates, will have mixing rates due to gardening that are similar or lower than Europa because of lower impact rate and velocity, both of which are controlled by the size of the parent planet and the planet-satellite distance.[25]

The effect of impact gardening to bury surface materials over short timescales is minimal on the icy bodies of our solar system.

Tensile Fractures

At the surface of an icy body, cold ice behaves as a brittle material. If the surface is subjected to tensile stress, the brittle material can fail, producing open tensile fractures. The abundance of cracks and faults on the satellites of the outer solar system attests to this process playing an important role in their history.[26] In theory, loose surface material could fall into open fractures, producing surface-subsurface material transport. Such a surface regolith drainage mechanism has been hypothesized to explain "pit chains" on Mars and Enceladus.[27,28]

The depth to which open tensile fractures can propagate from the surface is limited by the normal stress on the fracture imposed by the weight of the overlying ice. Below some depth the material fails in shear instead of tension, forming a sloping fault surface rather than an open crack. The exact depth of this transition depends on the strength of the brittle material and the surface gravity. The strength of pure, polycrystalline, unfractured laboratory ice falls within the range of 1 to 2 MPa, while ice in the natural environment has more mechanical defects and can be one or two orders of magnitude weaker.[29,30] The surface gravity of an icy body scales with its radius and its ice/rock ratio. As a consequence of these factors, open fractures can propagate on the order of hundreds of meters into the surface of a large icy body or a few kilometers into a mid-size icy body.

Cryovolcanism

In cryovolcanic eruptions, watery mixtures move from the interior of an icy body to the surface. Cryovolcanism has no direct terrestrial analog but appears to have occurred on several moons of the outer solar system, including Europa, Ganymede, Enceladus, Titan, Ariel, and Triton. The physical processes that enable the eruption of cryomagma from the interior of icy satellites remain unknown and may vary considerably from one satellite to the next.[31] Unlike terrestrial volcanism, where the magma is usually buoyant relative to the surrounding crust, the greater density of liquid water relative to ice causes the cryolava to sink instead of rising to the surface. Several mechanisms that might overcome this difficulty include[32] gas exsolution following depressurization in fluid-filled fractures that propagate upward from the base of the ice shell,[33] explosive eruptions of sprays;[34] pressurization of liquid chambers in an ice-I shell;[35,36] and pressurization of the entire ocean due to thickening of the ice shell.[37]

Surface temperatures on icy bodies are very low compared to the freezing temperatures of cryolavas. Although cryovolcanism represents interaction between subsurface liquids and the surface environment, material flows primarily toward the surface. From a planetary protection perspective, contact of a spacecraft with a cryovolcanic flow does not necessarily contaminate the source of the cryolava. Drain-back of surface lava that flows down eruptive fissures, possibly into the source chamber, is a rare event in terrestrial volcanism. It occurs in submarine volcanism in the East Pacific Rise[38] and on some basaltic eruptions such as those from Kilauea in Hawaii.[39,40] In both of these cases, lava lakes were present. Lava lakes are rare on Earth, and outside Earth they have only been observed on Io,[41] although past lava lakes may exist on Venus and Mars. In lava lakes, the subsurface melt conduit is hydraulically connected to the surface melt, so that decreases in pressure can result in a reversal of flow from the lake into the subsurface. There is no evidence that lava that has traveled downhill from a source vent could ever drain back to the interior. No features on icy bodies have been interpreted as cryolava lakes. It is possible that cryolava lakes do not form on icy bodies because the negative buoyancy of cryomagmas within icy crusts precludes the establishment of a stable lava lake. Therefore, we consider that drain-back events would be rare by comparison with Earth, and only topographic depressions surrounding active cryolava vents are of concern for planetary protection.

Near-Surface Melting

The negative buoyancy of liquid water within the ice crust suggests that if a mechanism existed to produce melt near the surface of an icy body, it could drain downward and provide an effective conduit for surface-subsurface transport. Concentration of tidal dissipation within the weakest, warmest ice in the cores of convective plumes could cause melting within the ice shell,[42,43] but not within the stagnant lid (see next section, "Convection"). Convective plumes within a relatively pure ice sublayer could induce melting within the overlying stagnant lid if other materials that lower the melting temperature, like salts, contaminate the lid.[44,45] Nimmo and Giese modeled this process for Europa and could not produce significant melt within 7 km of the surface using convective plumes.[46] However, even the production of a small amount of partial melt could contribute to enhanced tidal heating and plastic yielding near the surface[47] or infiltration of preexisting cracks in the near-surface brittle ice as the trapped, pressurized fluid follows hydraulic gradients within the ice shell.[48]

Another mechanism for near-surface melting is the concentration of heat flow from the interior of the body in sufficient amounts to thin the overlying ice shell. Once the shell has thinned beyond the thickness required for convection, heat conduction through the shell can control the thickness. Sufficiently concentrated heat could melt through the ice, thus exposing liquid at the surface,[49] but this requires more than 300 W/m^2 of heat flow from the interior.[50] Whether such a concentration of heat is even possible on an icy body is debatable;[51] for reference, this is a factor of 10^3 higher than the heat flow in the south polar terrain of Enceladus, the most geologically active known region on an icy body.

Convection

Like rocks in Earth's interior, water ice behaves as a fluid over geologic time scales. Radiogenic heating at the bottom or within the ice shells of icy bodies would warm ice at the base and cause it to rise from thermal buoyancy while cold ice sinks. Convection likely occurs in the outer ice shells of the large icy bodies and in smaller, tidally heated satellites.[52,53,54] The downward flow of cold ice would provide a pathway for relatively rapid transport to the ocean. Typical flow velocities of centimeters per year would yield a time scale for transport to the base of the ice shell (tens to a hundred kilometers) of ~10^5 years.[55] Because the surfaces of the outer planet satellites have low effective temperatures (~50 K to 130 K) and the viscosity of ice is strongly temperature-dependent, convective plumes are typically confined to a sub-layer of the ice shell beneath a "stagnant lid" of cold ice that is too stiff to participate in convection. Heat must be conducted across the stagnant lid. The stagnant lid serves as a barrier to mass transport between the surface of an icy moon and the convective sublayer. However, endogenic resurfacing processes on icy bodies could conceivably breach the stagnant lid, providing a means of communication between the surface, the convective sublayer, and the ocean.

The surface morphologies of Europa and Enceladus and observations of the high heat flow on Enceladus imply that convective motions have reached the surfaces of these bodies.[56,57] If the near-surface ice has an extremely low yield stress ~0.01 MPa,[58] it can be dragged along by the underlying convective motions. This style of convection, dubbed "sluggish" or "mobile-lid" convection is associated with a very thin layer of cold ice at the surface, which can locally achieve essentially zero thickness and, in some cases, periodically rip and sink to the base of the ice shell.[59] The predicted heat flow and resurfacing rates from sluggish lid convection within the Enceladus south polar terrain match estimated values,[60] lending support to the existence of this style of convection on tidally flexed icy moons, but perhaps only for short periods of time.

The thickness of the stagnant lid is important for determining the likelihood and timescale of transport across the geophysical "no-man's land" between the base of the stagnant lid and materials near the surface. A rough estimate of this thickness can be obtained by equating the radiogenic heat flux F_r to the convective heat flux F_{conv}. For large icy bodies, F_r ~ 5 to 10 mW/m^2. Tidal heating on Europa and Enceladus dwarfs radiogenic heating. The surface heat flux from tidal dissipation on Europa is plausibly

10 to 100 mW/m^2.[61] Estimates of the power output in the south polar terrain of Enceladus from Cassini CIRS data are currently 15.8 ± 3 GW,[62] which, spread over the 70,000 km^2 area of activity,[63] is equivalent to 225 ± 42 mW/m^2. The convective heat flux F_{conv} is related to the physical properties of the ice shell,[64,65] and the maximum F_{conv} will give the minimum thickness of the stagnant lid, δ_L. The minimum stagnant lid thickness occurs when the ice shell is so strongly heated that the temperature in the convective sublayer *is close to* the melting point of water ice. This situation likely arises on tidally heated icy satellites where the tidal deformation of the ice shell causes solid friction, which converts to heat in the warm interior of the shell.[66] If more heat is pumped into the shell, it will melt. For Europa, the maximum F_{conv} is 60 mW/m^2, within the range of possible tidal heat flows, and $\delta_L \sim 6$ km. For Enceladus, F_{conv} = 30 mW/m^2, which gives $\delta_L \sim 14$ km, while for the south polar terrain of Enceladus $\delta_L \sim 3$ km (assuming T_i = 273 K). These models assume that the viscosity and thermal conductivity of the shell is not significantly modified by non-water ice contaminants. The range of possible contaminants has been insufficiently explored to date, and is a topic requiring further research.

OBSERVED GEOLOGIC ACTIVITY ON ICY BODIES

Icy satellites have experienced global endogenic resurfacing through tectonics, cryovolcanism, or solid-state flow. Large areal coverage of recent (in the past 1,000 years) resurfacing poses the greatest concern for planetary protection. Special attention must be paid to bodies where the zone of near-surface brittle deformation has joined with the underlying convective motion to drive global resurfacing (e.g., Enceladus, Europa, and, possibly, Ganymede). Assuming that the mission does not require the spacecraft to penetrate to habitable environments beneath the ice, planetary protection must consider whether any active geologic processes might have a 10^{-4} chance of promoting surface-subsurface exchange of material within 10^3 years of contacting the surface.

Crater counting provides the only currently available method to assess the ages of geologic features on the surfaces of icy bodies. This method has some uncertainties due to uncertainty in the flux of impactors over time in the outer solar system. To be conservative, surface ages from the youngest limit are used, according to the fluxes of Zahnle et al.,[67] which will provide an upper limit on the rate of current geologic activity on an icy body. Take, for example, a hypothetical icy body with a crater age of 10^8 years. A surface with this crater age could be produced by either patchy regional resurfacing that wipes out craters over an average period of 10^8 years or a global resurfacing event that occurred 10^8 years ago. Assuming that resurfacing processes on this hypothetical body occur randomly in either space or time, there is a 10^{-5} chance that they would affect any particular area of the body in a 10^3-year period. Thus, an icy body with a surface age exceeding 10^8 years provides sufficient confidence that the geologic timescale for delivery of surface materials into potentially habitable subsurface environments vastly exceeds the timescale of biological exploration. To narrow the field of icy bodies of possible concern, it is assumed that the bodies that have surfaces in which the youngest craters are endogenically resurfaced terrains older than 10^8 years pose no concerns for planetary protection. Icy bodies that exhibit resurfaced areas younger than 10^8 years require greater scrutiny.

Icy Bodies with Recent Endogenic Activity

Using the 10^8 year (100 million years) geologic activity cutoff, the inventory of large and mid-size icy bodies can be divided into bodies that present no planetary protection concerns, and bodies that require closer examination before answering Decision Point 5. Almost all icy bodies are heavily cratered and easily fall into the "no concern" category. A few bodies lie near the border, but are still on the "no concern" side, including the following:

- Ganymede underwent widespread resurfacing over two thirds of its surface due to tectonism and, possibly, cryovolcanism.[68] The youngest limit for the crater age of these resurfaced areas occurred at 1 billion years.[69]
- Dione has areas recently cut by faults but exhibits no evidence of other types of geologic activity. Crater counting on these fractured plains indicates they could be as young as 260 million years.[70]
- Miranda's coronae appear to have formed primarily by tectonism, although they also exhibit unexplained albedo variations.[71,72] Two of Miranda's three resurfaced coronae exhibit young crater ages, which could be as young as 100 million years.[73]

After eliminating the icy bodies with no evidence for resurfacing activity in the past 100 million years, the committee only considered four icy bodies for planetary protection: Europa, Enceladus, Titan, and Triton.

Europa

The global average surface age of Europa could be as low as 20 million years or as high as 200 million years.[74] Crater ages for individual terrain units are difficult to reliably obtain because there are too few large craters for good statistics and the imaging datasets from Galileo and Voyager are insufficient to map the more abundant small craters, except for a few small target areas. One broad comparison of crater differences across classes of terrain types found that chaos terrain areas, which appear to be some of the youngest geologically resurfaced features based on crosscutting relationships,[75] have a higher crater density than the background ridged plains,[76] an apparent paradox. In the absence of reliable age information for any subarea of Europa, the age of the entire surface must be considered as a whole. Phillips et al. set an upper limit of 1 km^2/yr on the current surface modification rate, based on a lack of observable surface changes over 20 years.[77] This upper limit rate, which would lead to a lower limit estimate of 30 million years to resurface Europa, is broadly consistent with the lower limit surface age based on crater density of 20 million years.

If Europa has a relatively constant rate and style of resurfacing, and if any type of resurfacing would lead to the introduction of surface materials into a subsurface habitable environment, then the likelihood that a particular part of the surface undergoes resurfacing within 10^3 years is less than 5×10^{-5}. A consideration of the variation in resurfacing styles may further lower this likelihood. Two main styles of endogenic modification dominate Europa: resurfacing by the formation of ridges and bands and resurfacing by the formation of chaos terrain.

Several proposed models for ridge formation have different implications for near-surface habitability and communication with an underlying ocean.[78] At one end of the spectrum, the tidal pumping model posits that water from the ocean constantly travels up and down through cracks as they open and close during the diurnal tidal cycle.[79] This model requires a very thin ice shell for sufficient isostatic rise of ocean water into the crack, which is not supported by the record of large preserved impact craters.[80,81] At the other end of the spectrum, the linear diapirism model posits that ridges form in the solid state.[82] In between these end-members, a recent model of shear heating predicts formation of transient pockets of melt in the near-surface environment.[83]

Chaos terrain covers approximately a quarter of Europa's surface[84] and, like ridges on Europa, several proposed models explain its formation.[85] The most successful models of chaos formation involve some component of near-surface melting,[86,87] but the primary difference is whether convective plumes in the solid state (in which case the melt is trapped near the surface) or massive thinning of the ice shell (in which case near-surface melts may communicate with the ocean) drives the process. In either case, chaos terrain almost certainly signals the existence of greater amounts of liquid water near the surface than ridge formation. "Plates" of preexisting material that do not appear to have chaotically modified surface materials cover a substantial proportion of the area of chaos terrain.

Even if liquid water is produced within the ice shell as a result of chaos or ridge formation, it does not automatically indicate contact between surface materials with that liquid. For example, in the Nimmo and Gaidos ridge model,[88] the melt pockets develop a few kilometers below the surface. In the Schmidt et al. chaos formation model, briny melt is produced three or more kilometers below the surface. As the surface topography subsides over a liquid area, hydraulic gradients can push melt closer to the surface.[89] The cold, near-vacuum environment of the surface presents a hostile environment for the stability of liquid water, which means that melt that reaches the surface is on a one-way trip. Other than the intentional use of a perennial heat source sufficiently powerful to actively melt through Europa's ice, it is unclear what processes may be able to bury a spacecraft component deep enough to interact with circulating melt, especially considering that sputtering erosion of the surface by energetic particles is faster on Europa than burial by impact gardening.[90] It is also important to note that while the Schmidt et al. model predicts the existence of some melt in areas of the near subsurface, the model also requires that these melt bodies are sealed within the ice shell and do not communicate with the underlying ocean.

The prime concern for forward contamination on icy bodies is by organisms that can propagate at temperatures near the freezing point within the ice shell and the ocean. Europa's rocky interior may contribute a substantial fraction to the overall heat flow, which may lead to the existence of hydrothermal vents hosting steep temperature gradients suitable for organisms with higher growth optima (including mesophiles, thermophiles, and hyperthermophiles). Many thousands of hydrothermal systems might exist on Europa, distributed around the globe.[91] To get to a hydrothermal system, an organism introduced to the top of the ocean would need to sink to the bottom, enter a pore in the ocean floor, and then travel through cracks and pores beneath the ocean floor to an area of heating. Theoretical work on the timescale of vertical overturn in Europa's ocean is sparse, and may depend on poorly constrained salinity levels in the ocean.[92] If vertical mixing is driven by upward mass flux from hydrothermal plumes, it may take centuries for cold water at the top of the ocean to cycle to the bottom, and the time it takes for all of the ocean water to cycle through hydrothermal systems is ~10^7 years.[93] Given the low probability discussed above of accidental contact between materials on the surface and the water at the top of the ocean, the likelihood of non-psychrophilic contaminants reaching and colonizing a putative hydrothermal vent within 10^3 years is necessarily much lower.

In summary, a spacecraft in contact with a randomly selected portion of the uppermost surface of Europa is near the threshold of planetary protection concern as determined by application of Decision Point 5. That is, the probability of the spacecraft, spacecraft parts, or contents contacting a potentially habitable region within 1,000 years is uncomfortably close to the limit of 10^{-4}. The upper limit likelihood of 5×10^{-5} for chance contact between the surface and subsurface liquids (see above) within 1,000 years assumes constant resurfacing that always involves liquid water in contact with the surface. Such liquids must be cold brines hospitable only to psychrophilic organisms. There is currently active debate over the existence of liquids in contact with the surface, how widespread such liquids are, and whether such liquids communicate with the underlying ocean. Therefore this upper limit appears to be very conservative. However, Europa's activity may be episodic, in which case the constant resurfacing assumption may severely overestimate or underestimate the current situation. In the case of a mission that is intending to land on an area of suspected current resurfacing activity, the likelihood of chance contact with subsurface liquids is much higher. For a mission designed to penetrate the ice shell, the chance is higher yet. Such lander and penetrator missions would not pass the test imposed by Decision Point 5.

Enceladus

Despite its small size (mean radius 252 km), Enceladus is one of the most geologically active bodies in the solar system. Active cryovolcanism in the form of plumes occurs at the south pole,[94] coinciding with a concentrated thermal anomaly,[95] with an estimated thermal emission of 15.8 ± 3.1 GW.[96] The south polar terrain (SPT) is disrupted by tectonic features with almost no superposed craters, indicating a resurfacing age within the past million years.[97] Active venting of water vapor and icy particles is observed from four prominent fissures (commonly known as "tiger stripes") in the center of the SPT.[98,99,100] The temperatures near the source of this vented material exceeds 180 K,[101] while the presence of salts within the ejected particles implies that the plumes emanate from a subsurface liquid water source that has been in contact with the rocky interior.[102]

Nimmo and Pappalardo proposed that a solid-state convective ice under the south pole could explain the localized geologic activity of Enceladus.[103] Collins and Goodman showed that the geologic activity could also result from localized thinning of the ice shell over an isolated sea under the south pole, with the remnant ice about 9-km thick.[104] Tidal heating localized in a thermal plume could partially melt the ice shell and produce the high surface temperature of the south polar regions.[105,106] The observed tidal heating requires a subsurface ocean decoupling the ice shell.[107] Convection in the solid-state portion of the ice shell beneath the SPT may be vigorous enough to bring it into the mobile-lid regime, possibly recycling surface materials back into the interior.[108]

For the purposes of planetary protection, the south polar terrain on Enceladus presents a worst-case scenario with respect to Decision Point 5. Active venting from fissures in the ice may lead directly downward into a liquid water environment. The water is salty, indicating chemical reactions with the rocky interior and possible sources of nutrients. Unlike the other three icy bodies examined in this section, Enceladus' low gravity leads to very low normal stresses at a given crustal thickness, so cracks at the surface may stay open to a few kilometers depth. Alhough the chances of a spacecraft accidentally falling into one of the four narrow active fissures is quite small, caution is demanded because of the overall youth of the SPT, the countless number of tectonic features older than the "tiger stripes" (perhaps formerly active sites), and the possibility for surface recycling through mobile-lid convection. The south polar terrain of Enceladus does *not* pass the test of Decision Point 5, so any mission intending to travel to this terrain must meet additional planetary protection requirements and other missions in the vicinity must assess the probability of accidentally crashing into this terrain.

Areas outside the SPT have higher crater retention ages and do not appear to have been active within the past 100 million years. In particular, the cratered plains (which stretch from the subsaturnian point and over the north pole to the antisaturnian point) appear to have very little resurfacing activity within the past 1 billion years, besides a few narrow tectonic fractures that have permitted loose regolith drainage.[109] The cratered plains are of little concern from a planetary protection standpoint.

Titan

Titan is the only icy body with a dense atmosphere, and present-day surface-atmosphere interactions make aeolian, fluvial, pluvial, and lacustrine processes important on a scale previously seen only on Earth. Similar to Earth's water cycle, the activity on Titan's frigid 95 K surface is driven by a methane cycle of evaporation, precipitation, and runoff. The average crater surface age is between 200 million years and 1 billion years, which would remove Titan from the list of planetary protection concern,[110] except that there is evidence of some types of geologic activity in the present day, so it must be scrutinized.

The highest density of craters on Titan exists on the mountainous Xanadu region, while no craters are superimposed on the equatorial dunes,[111] indicating that they could be active. Other active processes observed on Titan include rainstorms[112] and changing lake levels.[113] Surface-atmosphere exchange processes drive all of the unambiguous recent geologic activity on Titan, and these processes do not provide a means of transport to liquid water environments habitable by terrestrial organisms.

From a planetary protection standpoint, the important question is where liquid water environments could exist within Titan and whether transport processes leading from the surface to those environments are currently active. Relevant to this point is the debate about the role that cryovolcanism may play in shaping Titan's surface, whether in the form of degassing or active flows. Before the Cassini-Huygens mission, various workers suggested that Titan is probably cryovolcanically active, on the basis of geochemical and geophysical models. Substantial quantities of ammonia in the interior could facilitate the melting that would lead to this cryovolcanic activity. Explanations for the present atmospheric abundance of methane require a replenishing mechanism, and cryovolcanism would provide a way to bring methane from the interior to the atmosphere. Cassini results, such as the detection of ^{40}Ar in the atmosphere,[114] support the case of cryovolcanism on Titan, as it implies outgassing from Titan's interior. Various features observed by Cassini on Titan have been interpreted as evidence of cryovolcanic activity. Putative cryovolcanic features were detected by Cassini's VIMS and radar instruments,[115] including lobate flows[116] and a tall mountain adjacent to a deep pit with lobate flow-like features.[117,118,] However, as Cassini acquired more data, particularly topographic data, the cryovolcanic origin of some of these features has come into doubt. It is often difficult to distinguish between fluvial, mass wasting, and volcanic flows using the relatively low-resolution data that are currently available from Cassini, and a self-consistent picture of Titan's geology can be constructed without any cryovolcanism.[119]

For the purposes of this report, the committee assumed that cryovolcanic activity on Titan remains a possibility. The area covered by putative cryovolcanic flows is 0.6 to 1 percent of Titan's surface, and the area of the source vents, where subsurface-surface transport takes place, is about a factor of 10 smaller. If it is assumed that all of Titan's surface is cryovolcanically resurfaced over the minimum surface age of 200 million years, and activity is randomly distributed in time and space, then the chance of a spacecraft randomly landing in the immediate vicinity of a source vent active within the next 1,000 years is less than 10^{-6}; comfortably within the bounds set by Decision Point 5. However, the flow of liquid methane across Titan's surface and through its regolith could pick up small particles from a spacecraft and carry them to lower elevations. Underground flow could move liquid methane from the equator (at higher elevation) to the pole (at lower elevation) in a matter of centuries.[120] If a spacecraft were to come into contact with Titan's surface at high elevations, methane flow could significantly spread out the contamination "footprint."

The lack of experimental research data on physical interactions that could occur between underground methane (carrying the contaminants) and cryovolcanic liquid water in the subsurface warrants pointing out a few first principles. The temperature difference between these liquids is about 180°C; in terms of homologous temperatures, cryolava encountering a lake on Titan is similar to terrestrial lava pouring into the sea. The water is likely to freeze at the same time that the methane boils. One crucial difference from the terrestrial analogy is that water is not significantly soluble in methane, unlike water interacting with silicate melts. Instead, near-freezing water and methane may solidify together into a more stable clathrate. If a terrestrial microbe were to reach a liquid water body on Titan, the extreme conditions could exceed the organism's limits of habitability. High concentrations of ammonia are likely to exist in cryovolcanic fluids on Titan, and the ocean on Titan probably does not contact the warm rocky interior due to high-pressure ice phases. The interior may even be cold and incompletely differentiated, although information about Titan's interior structure is more difficult to obtain than for the other saturnian satellites.[121] In summary, despite active exogenic processes operating on the surface, the evidence does not indicate that environments habitable to terrestrial organisms currently exist near the surface of Titan. Therefore, currently conceivable missions to Titan would pass the test imposed by Decision Point 5, and require no further planetary protection measures. However, thorough cleaning of these spacecraft may be desirable for other reasons related to mission science, such as sensitive detection of complex organic molecules in the titanian environment.

Triton

Images of Neptune's satellite Triton from the Voyager 2 spacecraft in 1989, revealed evidence of resurfacing processes,[122] possibly by cryovolcanism[123] and diapirism.[124] Triton's retrograde orbit around Neptune suggests that it is likely to be a captured satellite[125] and represents our best current model for a Kuiper belt object. Images revealed eruptive plumes up to 8 km high blowing dark particles downwind in the thin atmosphere.[126,127] Smith et al. suggested that solar heating and subsequent vaporization of subsurface nitrogen may drive the eruptions.[128] Other proposed mechanisms for gas venting include solid-state greenhouse[129] and convection in the solid nitrogen caps.[130] Therefore, current eruptive activity on Triton most likely reflects solar heating rather than endogenic cryovolcanism, in the sense of bringing molten material from the interior to the surface.[131]

The scarcity of impact craters on Triton indicates a surface younger than 50 million years in the oldest areas and only a few million years old in the youngest areas.[132] A number of geologic features on Triton suggest that widespread cryovolcanism has occurred in the past. Smooth plains with lobate features cover large parts of the observed surface, and interpretations of circular features termed "cantaloupe terrain" suggest they result from solid-state diapirism.[133] A possible explanation for the high heat flow required to drive this massive resurfacing is the orbital capture of Triton into the Neptune system followed by a period of high tidal heating and melting of the interior ices,[134] making it likely that Triton has an internal ocean.

A major factor for assessing the forward contamination of Triton is that the composition and the temperature of any subsurface liquids that could provide transport down to the ocean are not known. The surface of Triton is cold enough to host ices of nitrogen, methane, and carbon monoxide, and it is likely that ammonia may be mixed with the water ice in the crust.[135,136] There is a strong possibility that near-subsurface liquids on Triton accessible to a crashed spacecraft are uninhabitable by terrestrial organisms. But, in the absence of more information on the liquids, the combination of the very young surface age and active cryovolcanic processes strongly suggests that missions contacting the surface of Triton are likely to fail the test imposed by Decision Point 5. Therefore, Triton should be approached with caution from a planetary protection standpoint until more information is available.

CONCLUSIONS AND RECOMMENDATIONS

Planetary protection should focus on icy moons in the outer solar system where the preponderance of geophysical and chemical data indicates potential habitability for terrestrial life and where evidence of resurfacing activity in the past 10^8 years increases the likelihood of surface-subsurface transport to interiors that might be habitable. The requisite chemical species required for terrestrial life include liquid water and the key elements carbon, sulfur, nitrogen, potassium, magnesium, calcium, and phosphorus, but currently available data is not informative about their presence or absence on icy bodies. The physical conditions of the target body (e.g., temperature) must be compatible with extremes tolerated by terrestrial organisms.

> ***Recommendation:*** **Evidence of widespread resurfacing activity within the past 10^8 years requires that NASA evaluate planetary protection requirements for Europa, Enceladus, and Triton using a hierarchical decision-making framework of the kind presented in Chapter 2 and elaborated on in Chapter 3. Spacecraft designers must demonstrate that their plans for missions to these bodies have less than a 10^{-4} chance of contacting within the next 1,000 years an area of active surface-subsurface transport.**

> ***Finding:*** The possibility for active transport of contaminants into a habitable portion of Titan's interior over a 1,000-year timescale is more remote than 10^{-4}, removing Titan from high levels of concern for planetary protection. Titan's average surface age appears to be older than 10^8 years, and although putative cryovolcanic features have been found, all firm evidence for current geologic activity on Titan indicates that such activity is driven by exogenic processes involving the methane cycle and wind-blown sediment, none of which provides a habitable environment for terrestrial organisms.

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5
Microbial Metabolism and Physiology

The vast repertoire of metabolism and physiology allows different kinds of terrestrial microbes to colonize diverse environments. Because of niche competition, individual taxa have evolved to grow optimally under a limited range of conditions. For example, microbes that grow optimally at −15°C do not survive at 122°C, the known upper temperature limit for terrestrial life. Decisions about planetary protection must consider the interplay between availability of water (Decision Point 1), bioavailability of trace elements and sources of energy (Decision Points 2 and 4), microbial metabolism and physiology (Decision Points 2, 4 , and 6), the techniques used to reduce bioloads (Decision Point 7), and the environment of the target bodies (Decision Points 1, 2, 3, 4, and 6). Geophysical considerations (Decision Point 5) are less relevant. All require knowledge about the following:

1. The physical and chemical environment of the target body (Chapter 4);
2. The environmental source of organisms on the spacecraft and their ability to survive and grow at temperatures found on icy moons (e.g., at 0°C or below);
3. The relationship between growth at low temperatures and tolerance to heat-mediated bioload reduction; and
4. The survival tactics of microbial life in response to high levels of radiation or extremely low vacuum, which causes desiccation.

In addition to Decision Points 1-7, the location of assembly and launch facilities will influence planetary protection concerns. The cold environments of icy bodies might provide habitats for terrestrial microbes that can grow only at very low temperatures. On Earth, environments capable of supporting microbial growth at 0°C or below typically include temperate and high-latitude marine environments, high-latitude ice, soils, cryopegs, the upper atmosphere, seasonally cold soils, and dairy products, meats, and seafoods that are maintained at low temperature. Because of the location of spacecraft assembly facilities in the United States, soils from temperate environments are the most likely source of spacecraft contamination with an organism capable of growing on an icy planetary body. These organisms would have to grow at 0°C or below on the target bodies and also survive temperatures and other conditions involved with the assembly and launch of the spacecraft. A study of the cultured microorganisms from the spacecraft assembly facility at NASA Kennedy Space Center did not detect psychrophilic or facultative psychrophilic microorganisms or high-salt-tolerant organisms.[1] The predominant groups of organisms isolated included thermophiles, acidophiles, and ultraviolet-C- and H_2O_2-resistant bacteria. Molecular studies based on rRNA sequences and shotgun metagenomic analyses (enabled by anticipated improvements in DNA sequencing efficiencies) of low-biomass samples would provide a cultivation-independent assessment of microbial community composition within spacecraft assembly facilities and within and on a spacecraft.

DECISION POINTS 1, 2, AND 3

Decision Point 1—Liquid Water

The absolute and unambiguous requirement of liquid water for the propagation of terrestrial organisms on Earth or on icy bodies in the outer solar system constrains the possibility of forward contamination to a relatively small number of objects in the outer solar system. See Chapter 4 for a detailed discussion of this point.

Decision Point 2—Key Elements

The origin and development of life are intimately linked to the periodic table of elements. The most important elements required by living systems for catalysis, organization of macromolecular structure, or energy transduction include, but are not limited to, carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur, potassium, magnesium, calcium, and iron. Sometimes other elements such as boron participate in chemical signaling between bacteria,[2] or elements like selenium can incorporate into specific proteins as selanocysteine, now referred to as the twenty-first essential amino acid.[3]

Because of its pervasive role in many biological processes, phosphorus plays an indispensible role in living systems. In some marine organisms, arsenic can incorporate into lipids in place of phosphorus,[4] but this generally toxic compound does not replace phosphorus in nucleic acids, in protein structures, or in catalytic functions. There is no consensus as to the minimum concentration of phosphorus required for growth by microorganisms. The cultivation of different bacterial taxa in phosphate at varying concentrations reveals a complex set of genes that mediate stress-response to phosphate limitation. In general, phosphorus limitation triggers adaption, including the up-regulation of specific genes involved in phosphate response stress that shift cells from utilization of inorganic phosphate to scavenging of organo-phosphate and polyphosphate from the environment,[5,6] substituting sulfur for phosphate in membrane lipids in marine photosynthetic bacteria,[7] changes in cell morphology to increase surface-to-volume ratio of the cell,[8] and shutting down cell metabolism to survive in a dormant stage.[9,10,11] In most cases, these studies were preformed with bacteria that grow in high concentrations of organic nutrients, and the concentrations of phosphorus at which the stress response is stimulated are considerably higher than those measured in oligiotrophic oceans (0.2 to 1 nM inorganic phosphate). Even in studies with the freshwater oligiotrophic bacterium *Caulobacter crescentus,* 30 µM of phosphate induced an adaptive response.[12] In contrast, various strains of *Rhizobium* species, a nitrogen-fixing soil microbe, grew as rapidly at 0.05 µM phosphate as at 2 mM.[13] The data on the effects of phosphate limitation on spore-forming microorganisms includes studies only with the mesophiles *Bacillus subtilis* and *Clostridium perfringens*. In *B. subtilis*, concentrations of phosphorus below 0.1 mM led to reduced growth rates and entry into stationary growth phase,[14,15] whereas in *C. perfringens*, sporulation did not occur when phosphorus concentrations were less than 3 mM.[16] There were no studies found that looked at the effects of phosphorus limitations on psychrophilic or facultative psychrophilic spore-forming bacteria.

The studies on phosphorus limitation using pure cultures of microorganisms have revealed the exquisite complexity of physiological responses and survival mechanisms. However, complex ecosystems exist in oligiotrophic environments where the concentration of phosphate is lower than the lowest concentration that either prevents growth or induces stress responses in most isolated microbes tested. Oligiotrophic oceans such as the Sargasso Sea in the northwestern Atlantic, the North Pacific subtropical gyre, and eastern part of the Mediterranean Sea have extremely low levels of dissolved phosphate. For example, the concentrations of phosphate in surface waters of the subtropical Sargasso Sea are from 0.2 to 1.0 nM.[17,18,19] The canonical "Redfield ratio" used in biogeochemical models of the ocean is 106C:16N:1P and does not apply to oligiotrophic oceans where the N:P ratio can be higher than 30.[20] Although these environments show limited phosphate stress, they have active ecosystems anchored by *Prochlorococcus spp.* as the primary producers. These photosynthetic bacteria are highly adapted to low levels of nutrients, including phosphate, and can compensate by synthesizing sulfur lipids instead of phospholipids.[21] It was further observed that the synthesis of membrane lipids normally accounted for 18 to 28 percent of the phosphate utilized by phytoplankton. The dominant heterotrophic bacteria in these oligiotrophic oceans, *Pelagobacter ubique*, can like the *Prochlorococcus* species grow in situ in phosphate at low concentrations while utilizing carbon compounds in the low levels found in the dissolved organic compound fraction.[22] Both of these organisms are small (<1 μm) and have the smallest genomes of "free-living" organisms and are genetically surprisingly well adapted to grow in the presence of phosphate and other inorganic nutrients at ultralow concentrations. It is apparent that microorganisms have adapted to phosphorus limitations and, in particular, have developed the ability to grow in phosphate at concentrations that are at the edge of researcher's ability to measure. Consequently, it might not be possible to measure phosphorus in the oceans or ice of icy moons if it is present only at the low levels measured in present day oligiotrophic oceans.

Decision Point 3—Physical Conditions

Radiation flux and temperature extremes that exceed the documented limits tolerated by life on Earth will constrain the potential growth of terrestrial microbial life forms in target body environments. These same parameters have important implications for Decision Points 6 and 7, which consider the survivability of irradiated microbes in oligotrophic environments and at temperature in ranges in which cold-loving organisms can survive.

DECISION POINT 4—CHEMICAL ENERGY

Determining the minimal energy requirements for life is far more difficult than constraining the possibilities of contamination according to the presence or absence of water or essential elements. Discoveries each year of novel extremophiles with new types of metabolism continuously change understanding of life's minimal energy criterion and the chemical dimensions of Earth's "habitable zone." Two considerations suggest taking an optimistic approach to the presence of energy: (1) work in the fields of electron transport and metabolism in the past 20 years has made it clear that some group of terrestrial organisms has made use of nearly any redox couple that can yield significant energy and[23,24,25,26,27,28] (2) observational, laboratory, and theoretical studies show that icy bodies contain a vast number of oxidized and reduced compounds, many of which might serve as potential electron donors or acceptors (Table 5.1).

Researchers currently lack conclusive information about the presence of energy and elemental sources necessary to support the growth of potential contaminating organisms on icy bodies. Electron donors (e.g., Fe^{2+}, SH^{-}, organic carbon) and electron acceptors (e.g., CO_2, SO_4^{2-}, O_2, H_2O_2)[29,30,31,32,33] might be present on some icy bodies. Given the poorly constrained knowledge of the chemistry of these environments, a supply of energy cannot be ruled out. Here the committee takes the conservative assumption that if an environment contains essential elements and water, then a source of energy might exist.

DECISION POINT 6—COMPLEX NUTRIENTS

Radiation-resistant microorganisms might present a special problem when the possibility of forward contamination is considered. "Stowaway" bacteria and archaea on spacecraft targeting the outer planets and their moons will experience exposure to high-level radiation.[34] Yet the extreme resistance of some bacteria on Earth to acute and chronic forms of ionizing radiation that feasibly mimic conditions during transit argues that some microbes within sealed spacecraft components might survive such a journey, because of their resistance to gamma rays afforded by biochemical properties and/or by microenvironments of biofilms, and ultimately could reach life-supporting environments.[35,36,37] For example, as the level of cell grouping increases, survival characteristics of irradiated organisms typically increase as a result of the effects imposed by the limitation of atmospheric dioxygen, whereby cells within biofilms or within clumps are shielded from oxygen effects. Hence, prokaryotes under oxidative stress tend to adhere to surfaces and to each other during growth.[38] Given the possibility that such organisms might survive irradiation exposure and come into contact with a habitable environment, efforts to prevent forward contamination must consider the ultimate fate of these potentially viable organisms on icy moons.

TABLE 5.1 Examples of Electron Donors or Acceptors for Life in Icy Bodies Both Inferred and Measured in Past Work

	Europa	Comments	Enceladus	Comments	Titan	Comments
Electron Donors (observed)						
Organics	Detected on surface of Callisto, Ganymede Reference a	Expected on the surface. Interior unknown	Organic compounds Reference j Size of some known; structures not known	Methane and other organics detected in the plume.	Organics and methane Reference l and m suggested as a source of energy for organisms by references n and o	Surface organics from hydrocarbon cycle
Hydrogen	n/a	n/a	n/a	n/a	Reference o	
Electron Donors (suggested in literature as plausible candidates)						
Hydrogen	Reference b		Reference k			
Electron Acceptors (observed)						
Oxygen	Observed on surface Reference c	Interior unknown				
CO/CO_2	CO_2 on surface Reference d		Reference j	Observed in plume; CO and N_2 peaks cannot be differentiated	Trace quantities of CO_2 observed Reference o	
SO_2	Reference e					
Electron Acceptors (suggested)						
Sulfur	Sulfate inferred on surface Reference f and g Elemental sulfur also suggested Reference h	Interior unknown				
Fe^{3+}, other metals	Fe^{3+}, other metals Reference b, i	Fe^{3+}, other metals depends on connection with silicate core				

NOTE: Comments are provided as caveats and context.
[a] Hand, K. P., C. Chyba, J.C. Priscu, R. W. Carlson, K.H. Nealson, Astrobiology and the Potential for Life on Europa, pp. 589-630 in *Europa* (R.T. Pappalardo, et al., eds.). Univ. Ariz. Press, Tucson, 2009;
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[i] Gaidos, E.J., K.H. Nealson, and J.L. Kirschvink, Life in ice-covered oceans, Science, 284:1631, 1999.
[j] Waite, J.H. et al., Cassini ion and neutral Mass Spectrometer: Enceladus plume composition and structure. *Science*, 311, 1419-1422, 2006.
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[n] Fortes, A.D., Exobiological implications of a possible ammonia-water ocean inside Titan, *Icarus* 146:444-152, 2000.
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Unlike many terrestrial extremophiles that grow within a singular extreme environment, species of the genus *Deinococcus* demonstrate a suite of extreme survival advantages similar to that needed to survive multiple challenges encountered on missions to the outer planets. One of these species, *Deinococcus radiodurans*, can survive exposures to ionizing radiation (x rays and gamma rays), ultraviolet C (254 nm) radiation, and charged particles.[39,40] For example, *D. radiodurans* can survive exposure to 12,000 Gy of gamma rays in aqueous preparations; and notably, when desiccated in vacuo or when deeply frozen, *D. radiodurans* shows significantly increased resistance to gamma rays and ultraviolet C (Figure 5.1).[41,42,43,44] Thus, the psychrotolerant, desiccation-resistant *D. radiodurans* could survive the simultaneous assaults of impinging cosmic radiation (e.g., far-ultraviolet light and charged subatomic particles), drying, deep freezing, and, possibly, the exposure to high-level ionizing radiation anticipated during orbits that transect the radiation belts of Jupiter or Saturn.[45,46] There is, however, a prerequisite for recovery of *D. radiodurans's* enhanced DNA repair, a capacity that is absolutely dependent on the availability of a rich source of aqueous heterotropic organic growth substrates. Thus, the survival traits of *D. radiodurans* render it the best currently known model for estimating the outer limits of microbial survival in missions to icy solar system bodies.[47,48,49,50,51] Remarkably, the amount of DNA damage caused by most of the physico-chemical insults to which *D. radiodurans* is most notably resistant is about the same as that in radiation-sensitive cell-types.[52] For example, the yield of DNA double-strand breaks (DSB) in *D. radiodurans* caused by gamma rays is about the same as that seen in sensitive bacteria, simple eukaryotes, and animals (0.004 DSB/Gy/Mbp).[53,54,55] *Deinococcus* and other radiation/desiccation-resistant microbes rely on conventional DNA repair enzymes that function extraordinarily efficiently in those species.[56,57,58,59] Resistant bacteria and archaea have evolved potent chemical defenses that specifically protect their proteins from oxidative damage, thereby preventing inactivation of enzymes, including those needed to repair and replicate DNA.[60,61] Under this model, a single system rather than a series of separate repair mechanisms evolved to provide resistance to multiple stressors.[62]

The survival of *D. radiodurans* following genotoxic assault depends on the availability of fresh complex nutrients during recovery.[63,64,65] Under nutrient-poor conditions, metabolic capabilities limit DNA repair in acutely irradiated *D. radiodurans*,[66,67] and similarly in chronically irradiated *D. radiodurans*.[68] This nutrient-dependent phenotypic reversal from radiation resistance to radiation sensitivity alters scientists' view of radiation survival in the context of planetary protection. Figure 5.1 illustrates the effects of nutrient conditions and temperature on the recovery of *D. radiodurans* exposed to gamma radiation. Under standard laboratory conditions, *D. radiodurans* exposed to 12,000 Gy (from a

^{60}Co source) on wet ice (at 0°C) displays 10 percent survival (D_{10})[69] for radiation inactivation when recovered in liquid rich medium (TGY, tryptone/glucose/yeast extract) at 32°C;[70] the D_{10} for *D. radiodurans* irradiated at −70°C and recovered in TGY is 50,000 kGy.[71,72] However, if irradiated *D. radiodurans* cells are transferred to an aqueous solution of 10 mM $MgCl_2$ (without addition of complex carbohydrates, peptides, sugars, or vitamins), the D_{10} for radiation inactivation falls to 5,000 Gy within a few hours of incubation (Figure 5.1), followed by a progressive loss in viability.[73] It follows that the accumulating radiation doses in frozen, hence non-repairing, cells on spacecraft would eventually destroy them unless they were transferred to a liquid environment that contains a rich source of complex organic compounds. Similar arguments and observations apply to radioresistant *Bacillus* spores[74,75] and radioresistant archaea,[76,77] which are significantly less resistant than *D. radiodurans* (Figure 5.1). DNA repair in irradiated *Bacillus* spores occurs at the onset of germination, which requires heterotrophic organic substrates that would not be present in oceans or liquid water reservoirs of moons of the outer planets, and the most radioresistant archaea reported, *Halobacterium salinarum* NRC-1 and *Pyrococcus furiosus*, also are chemoorganotrophs.[78,79]

Thus, for radiation-resistant microbes, the probability of surviving exposure to radiation and their ability to grow are inextricably linked to the availability of growth substrates. For bacteria such as *D. radiodurans*, it is conceivable that a very small fraction of cells frozen at −70°C, or lower temperatures, could survive 50 to 80 kGy of gamma rays if recovered in complex organic media.[80] However, recovery of heavily irradiated *D. radiodurans* (or other resistant microbes) will not likely occur in simple salt solutions lacking complex organic compounds. For *D. radiodurans* and the radiation-resistant model archaea *H. salinarum* NRC-1[81] and *P. furiosus*,[82] exposure to ionizing radiation at doses in the range and context anticipated for missions to the outer planets would render them incapable of growth or recovery on icy bodies. Similarly, irradiated *Bacillus subtilis* and *Bacillus megaterium* spores will not survive, because germination with attendant DNA repair requires complex organic compounds.[83,84]

Generally speaking, the most radiation-resistant bacteria reported have been gram-positive (e.g., *D. radiodurans*), and the most sensitive have been gram-negative (e.g., *Shewanella oneidensis*). However, there are several reported exceptions to this paradigm, including the extremely radiation-resistant gram-negative cyanobacterium *Chroococcidiopsis*.[85] Cyanobacteria are phototrophs, which use the energy from sunlight to convert carbon dioxide and water into organic material to be utilized in cellular functions such as biosynthesis and DNA repair. Any potentially habitable environments of icy bodies would be located beneath the thick external ice I shell and are therefore inaccessible to sunlight. Thus, any terrestrial phototroph potentially able to survive transport to an icy solar system body would not survive there.

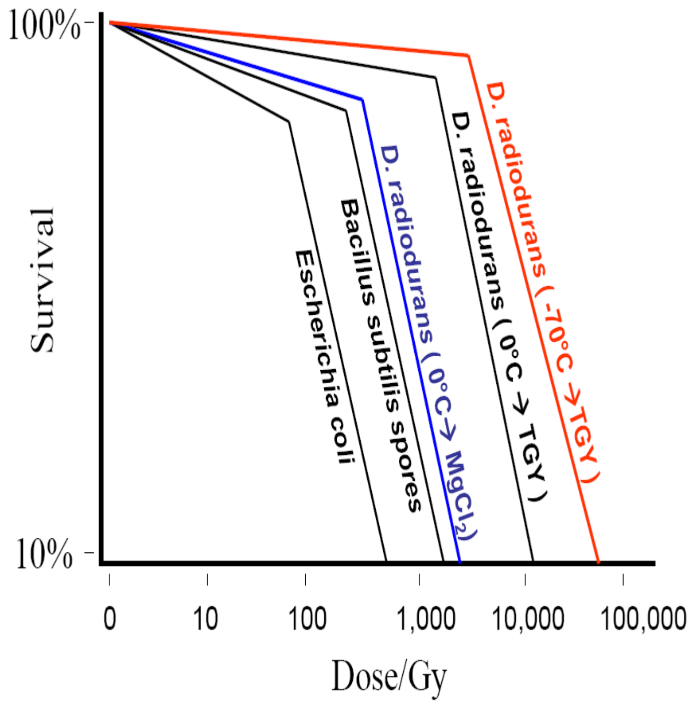

FIGURE 5.1 Gamma-radiation survival profiles of wild-type bacteria. *B. subtilis* spores and *Escherichia coli* were irradiated on wet ice (0°C) and recovered on rich medium (Granger et al., 2011; Daly et al., 2004). *D. radiodurans* was grown in rich medium (TGY) and treated as follows: −70°C→TGY: irradiated frozen on dry ice, then recovered on TGY (Richmond, 1999); 0°C→TGY: irradiated on wet ice, then recovered on TGY (Daly et al., 2004); 0°C→$MgCl_2$: irradiated on wet ice, then transferred to 10 mM $MgCl_2$ for 5 hours, then recovered on TGY (Ghosal et al., 2005). Note, the D_{10} radiation-inactivation values for the radioresistant archaea *P. furiosus* and *H. salinarum* NRC-1 are 3 and 5 kGy, respectively (DiRuggiero et al., 1997; Kottemann et al, 2005). SOURCES: Data from A.C. Granger, E.K. Gaidamakova, V.Y. Matrosova, M.J. Daly, and P. Setlow, Effects of Mn and Fe levels on *Bacillus subtilis* spore resistance and effects of Mn2+, other divalent cations, orthophosphate, and dipicolinic acid on protein resistance to ionizing radiation, *Applied and Environmental Microbiology* 77:32-40, 2011; Daly, M. J. Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Venkateswaran, A., Hess, M., Omelchenko, M. V., Kostandarithes, H. M., Makarova, K. S., Wackett, L. P., Fredrickson, J. K. and Ghosal D. (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* 306, 1025-1028; R.C. Richmond, R. Sridhar, and M.J. Daly, Physicochemical survival pattern for the radiophile *Deinococcus radiodurans*: A polyextremophile model for life on Mars, *SPIE* 3755:210-222, 1999; D. Ghosal, M.V. Omelchenko, E.K. Gaidamakova, V.Y. Matrosova, A. Vasilenko, A. Venkateswaran, H.M. Kostandarithes, H. Brim, K.S. Makarova, L.P. Wackett, J.K. Fredrickson, and M.J. Daly, How radiation kills cells: Survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress, *FEMS Microbiology Reviews* 29:361-375, 2005; J. DiRuggiero, N. Santangelo, Z. Nackerdien, J. Ravel, and F.T. Robb, Repair of extensive ionizing-radiation DNA damage at 95°C in the hyperthermophilic archaeon *Pyrococcus furiosus*, *Journal of Bacteriology* 179:4643-4645, 1997; M. Kottemann, A. Kish, C. Iloanusi, S. Bjork, and J. DiRuggiero, Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation, *Extremophiles* 9:219-227, 2005.

DECISION POINT 7—MINIMAL PLANETARY PROTECTION

Current data indicate that the outer solar system objects Europa, Enceladus, and Triton harbor liquid water-ice interfaces where persistent temperatures will not exceed 0°C or lower, with the possible exception of deep, interior, localized regions that lie in proximity to heat sources. Terrestrial organisms capable of growth at these low temperatures include obligate psychrophilic (i.e., cold-loving) microbes that have growth temperature optima below 0°C, and facultative psychrophiles (i.e., cold-tolerant microbes) also can grow below 0°C, but optimally at 20°C to 30°C. The lowest recorded temperature for growth is −15°C and for active metabolism is −20°C. Most of the characterized obligate psychrophiles are gram-negative aerobic heterotrophs, although there are a wide diversity of psychrophilic and facultatively psychrophilic gram-positive spore-forming and non-spore-forming aerobic and anaerobic bacteria and chemolithoautotrophic and anoxygenic photosynthetic bacteria. The following two aspects of microbial physiology have important implications for planetary protection:

1. Psychrophiles can grow only over a limited temperature range that does not exceed 20°C to 30°C, whereas the temperature range of growth for facultative psychrophiles is somewhat broader at 30°C to 40°C. Thus, for both kinds of physiology, the anticipated maximum growth temperature lies between −5°C and 40°C.
2. Non-spore-forming psychrophiles have short survival times at temperatures above their maximum growth temperature and in some cases lyse within minutes after exposure to temperatures a few degrees above their maximum growth temperature.[86]

Genomic and physiological characterization of cultured species of non-spore-forming psychrophiles shows a diverse range of strategies for growth and survival that can help them to compensate at low temperature but not for temperatures significantly higher than the upper temperature for growth. A single enzyme or group of enzymes for protein synthesis or energy generation can determine the upper temperature limit for psychrophiles or facultative psychrophiles.[87] Proteins adapted for optimal activity at low temperature are generally denatured in minutes at 50°C.[88] Similarly, the membrane lipids of psychrophiles have adapted to low temperature by lowering their fluidity, but these same modifications cause cell membranes to become leaky and nonfunctional at elevated temperatures. In some cases, the onset of this leakiness occurs minutes after exposure to temperatures a few degrees centigrade above the maximum growth temperature.[89,90]

Non-spore-forming psychrophiles will not survive short-time (minutes) exposure to temperatures greater than 20°C. Non-spore-forming facultative psychrophiles will not survive short-time exposure to temperatures above their maximum growth temperature (>20°C to 40°C). Similarly, vegetative cells of fungi and yeasts are generally killed within 10 to 15 minutes of exposure to temperatures of 50°C to 70°C. The overall conclusion is that psychrophiles and facultative psychrophiles are not adapted at the molecular level to grow or survive at temperatures much more than10°C above their maximum growth temperature. Therefore, to meet planetary protection requirements for missions to the icy bodies, heating of the spacecraft or its sealed components to 60°C for 5 hours will provide sufficient bioload reduction for non-spore-forming psychrophiles and facultative psychrophiles.

Spore-forming psychrophiles and facultative psychrophiles include heterotrophs that have complex requirements for organic compounds, and the few described species grow only under anaerobic conditions. These organisms have been isolated from high-latitude permafrost, soil and lake samples, temperate soils, and various animal and dairy products kept at low temperature. In most cases, information is lacking regarding the thermal resistance of spores from psychrophiles. For example, psychrophilic *Clostridium* species isolated from permafrost have optimum growth temperatures from 4°C to 16°C, with maximum growth temperatures near 20°C, but no discussion of the thermal properties of its spores exist.[91] The newly described isolate *C. algoriphilum* can survive for 24 hours at 20°C but cannot survive at 24°C.[92] The spores from six facultatively psychrophilic *Bacillus* species isolated from dairy products had D_{10}[93] values for heat inactivation of only 4.4 to 6.6 minutes at 90°C.[94] In contrast, spores from mesophilic *Bacillus* species had D_{10} values for heat inactivation of 70 to 200 minutes at 90°C. There appears to be a correlation between the maximum growth temperature of spore formers and the maximum temperature for inactivation of the spores. For 28 strains of *Bacillus* having different maximum growth temperature, Warth observed that the spores had a D_{10} value for heat inactivation of 10 minutes at approximately 40°C above the maximum growth temperature (Figure 5.2).[95] No psychrophilic *Bacillus* isolates were used in that study. In fact, most of the studies in which D_{10} values have been calculated are for spores exposed to high temperatures for short times.

Spores from psychrophilic bacteria are likely to be rendered inactive at 40°C above their maximum growth temperature, which corresponds to 60°C or lower. While there are few specific studies that discuss the length of time of exposure to spore-inactivating temperatures, most exposures are for short periods. For all obligate psychrophiles including spore-forming taxa, heating at 60°C for 5 hours provides a sufficient margin of error to achieve complete inactivation. This temperature and length of heating should pose minimal challenges for spacecraft design, including sensitive instrumentation. There is insufficient data to estimate a D value for spores of facultative psychrophile at 60°C, and additional research will be necessary to address this knowledge gap. Another factor concerns the nutritional requirements for spore-forming microbes to go from vegetative cells to spores and vice versa. Essentially, all of the spore formers are heterotrophic, and most have complex requirements for growth and for transitioning from spores to vegetative cells.

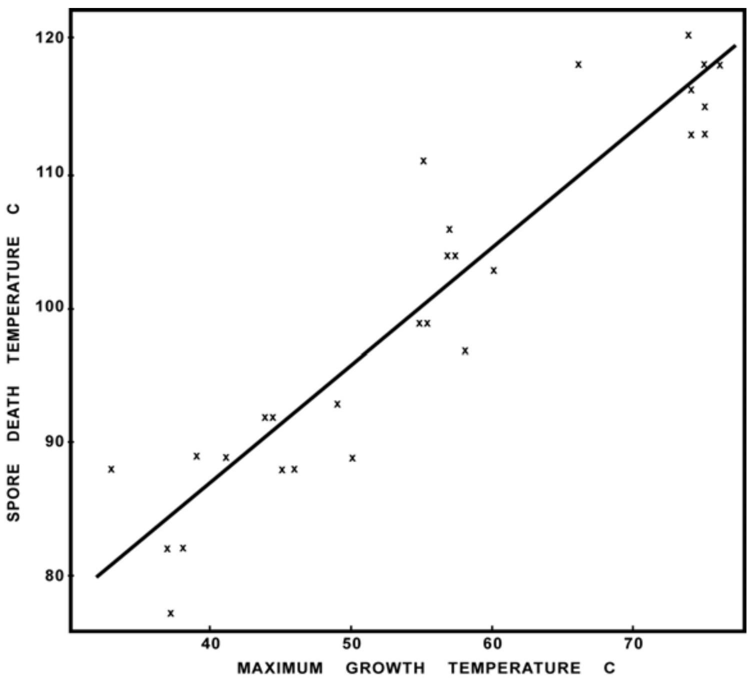

FIGURE 5.2 The spore death temperature (the temperature at which D = 10 minutes) in relation to maximum growth temperature for 28 strains of *Bacillus*. SOURCE: A.D.Warth, Relationship between the heat resistance of spores and the optimum and maximum growth temperatures of Bacillus species, *Journal of Bacteriology* 134:699-705, 1978.

CONCLUSIONS AND RECOMMENDATIONS

Beyond the absolute requirement for liquid water, life cannot propagate on icy bodies in the outer solar system in the absence of phosphorus or without redox couples for energy. Physiological capabilities of potential contaminants determined through metagenomic surveys of microbial taxa in component and spacecraft assembly facilities could impact many different aspects of planetary protection. Molecular surveys could determine the presence of cold-tolerant organisms and the identification of radiation-resistant taxa. However, the ability of organisms to resist the effects of exposure to radiation during flight or on icy bodies in the outer solar system would require the unlikely availability of complex heterotrophic organic substrates. Energy requirements also pose a challenge to the propagation of potential contaminants, but at this time, currently available data about redox-couples or the presence or absence of key elements is not sufficiently informative to guide planetary protection policies. In contrast, a growing body of evidence argues that psychrophiles grow over a limited temperature range of ~20°C, and most lyse within minutes after exposure to temperatures a few degrees above their maximum growth temperature. The few examples of cold-tolerant, spore-forming organisms that are known require complex organic compounds to grow. The preponderance of currently available data supports the view that heating at 60°C will be sufficient to inactivate spores from psychrophilic microorganisms.

> ***Finding:*** If the preponderance of data eliminates the presence of liquid water, the likelihood of bioavailable phosphorus, sources of redox-couples for energy, or complex organics required for radiation resistance on icy bodies in the outer solar system, planetary protection will require only routine spacecraft cleaning and minimal monitoring.
>
> ***Recommendation:*** **Molecular-based inventories of bioloads, including both living and dead taxa, must be collected in order to document the range of physiological capabilities of potential contaminants in component and spacecraft assembly facilities.**
>
> ***Recommendation:*** **If the probability of contamination by psychrophilic and facultative psychrophilic spore-forming organisms exceeds 10^{-4} after treatment at 60°C for 5 hours, full Viking-level, terminal bioload reduction procedures must be undertaken for planetary protection.**

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6
Necessary Research

The decision hierarchy outlined in this report provides a robust framework, but there are remaining uncertainties in the answers to several of the decision questions. The five research areas described below are important for addressing these uncertainties. The first three areas address the nature of the bioload on the spacecraft before launch, and the last two areas address environmental conditions on icy bodies.

HEAT RESISTANCE OF COLD-LOVING SPORES

Limited data exists on the heat inactivation of spores from psychrophilic and facultative psychrophilic bacteria. The general strategy for determining the inactivation of spores by heat or other treatment relies on the calculation of D values (inactivation of 90 to 100 percent of the spores) over some period of time for some specific treatment. With heat treatment using high temperatures (90°C or higher) a D value is generally attained in 10 minutes or less. Inactivation of spores will occur over different time intervals depending on the temperature. This kind of analysis, however, has not been done for psychrophilic and facultative psychrophilic bacterial spores.

> ***Recommendation:*** **The D-value times for heat inactivation of spores from psychrophilic and facultative psychrophilic spore-forming bacteria should be determined at different temperatures, specifically between 40°C and 80°C. These analyses should include psychrophilic and facultative psychrophilic bacteria isolated from high-latitude soil, water, and cryopeg samples, as well as facultative psychrophiles isolated from temperate soils, spacecraft assembly sites, and the spacecraft itself.**

> ***Recommendation:*** **Studies should be undertaken to better understand the environmental conditions that initiate spore formation and spore germination in psychrophilic and facultative psychrophilic bacteria so that these requirements can be compared with the characteristics of target icy bodies.**

> ***Recommendation:*** **Searches should be undertaken to discover unknown types of psychrophilic spore-formers and to assess if any of them have tolerances different from those of known types.**

ENHANCED RESISTANCE OF BIOFILMS

Biofilm growth confers greater than usual resistance to a diversity of environmental extremes,[1] and microbial functional redundancy in biofilms might also confer resilience to environmental extremes.[2] Future research can address the extent to which organisms within communities or biofilms may exhibit increased resistance to the high temperatures used for terminal bioload reduction. Although the exterior of a spacecraft that has been assembled in a clean room is unlikely to harbor communities within biofilms, the protected interior of spacecraft might contain microenvironments in which organisms are in contact and behave as biofilms.

Protected microenvironments within spacecraft have to be characterized, and their microbial ecology has to be assessed. Moreover, research is needed to determine whether biofilm growth of organisms associated with spacecraft microhabitats can influence their resistance to heat treatment and other environmental extremes encountered on journeys to icy bodies.

> ***Recommendation:*** **Research should be undertaken to characterize the protected microenvironments within spacecraft and to assess their microbial ecology.**

> ***Recommendation:*** **Research should be undertaken to determine the extent to which biofilms might increase microbial resistance to heat treatment and other environmental extremes encountered on journeys to icy bodies.**

IMAGING METHODOLOGY TO DETERMINE BIOLOAD

The long-standing NASA standard used to assess microbial contamination on spacecraft during assembly, test, and launch operations uses a Petri-plate-based culturable assay method to determine the number of cultivable aerobic bacterial endospores present on surfaces of interest. This assay takes 72 hours to complete, which can be extremely challenging and costly in a time-constrained hardware assembly environment. Because it relies on swab or wipe sampling, the assay method cannot be used directly for parts that cannot be touched or that are sensitive to the water matrix used for sampling. New techniques for obtaining real-time accurate assessments of microbial burden on flight hardware could provide a significant improvement over the current culture method.

The ideal solution would be a non-invasive, non-destructive technique that can be used to scan a spacecraft's surfaces and identify living microbes through detection of morphologies of cells that are alive, as indicated by the presence of ribosomal RNA transcripts. There are several techniques that might be stepping stones toward the goal of detecting individual microbes on a spacecraft: e.g., Raman and fourier transform infrared spectroscopy. However, some of these techniques, for example, lack a scanning capability or cannot distinguish between living and dead or metabolically inactive cells.

One particularly promising new technique that might be applicable to the assessment of the bioload on a spacecraft is deep-ultraviolet (224 to 250 nm range) imaging.[3,4] The advantage of using short-wavelength ultraviolet radiation is that most minerals and solid surfaces are non-fluorescent at this wavelength, whereas strong fluorescence is seen from the amino acids tryptophan, phenylalanine, and tyrosine, so that any organism containing proteins with these amino acids will be detectable by autofluorescence (i.e., without the addition of fluorescent stains or dyes). The identification of single cells through fluorescence scanning at low magnification can also provide a quantitative measurement of bioload. Real-time analyses of positive targets at higher magnification can enable identification of cells' morphological properties, including the ability to differentiate bacterial spores from vegetative cells.

The deep-ultraviolet imaging method should be applicable to the assessment of bioload on the surfaces of spacecraft. As shown in the adjacent images of Figure 6.1, bacteria "hiding" in the matrices of well-scrubbed surfaces of stainless steel are easily seen using deep-ultraviolet fluorescence imaging. In addition, because of their high tyrosine content (and probably other chemical differences), the spores have a fluorescent signal that differs from the signal of vegetative cells, making the approach valuable for direct determination of bacterial spores on surfaces. Low-resolution scanning could identify areas of fluorescence followed by high-resolution imaging to count cells.

Maturing and validating deep-ultraviolet fluorescence imaging to the level of automatic quantitative sampling of spacecraft surfaces would be a major positive addition in the area of planetary protection quality assurance.

Recommendation: **Technologies should be developed to directly detect and enumerate viable microorganisms on spacecraft surfaces.**

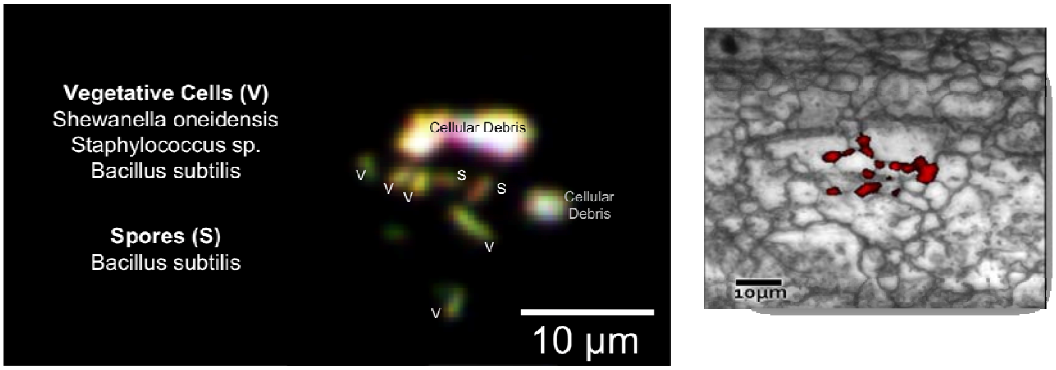

FIGURE 6.1 Vegetative cells and spores on the same petri plate (*left*); vegetative cells "hiding" on a plasma-cleaned surface (*right*). SOURCE: Images courtesy of R. Bhartia, Jet Propulsion Laboratory.

AVAILABILITY OF BIOLOGICALLY IMPORTANT ELEMENTS

The availability of key elements necessary for life (carbon, hydrogen, oxygen, nitrogen, and phosphorus, as well as trace nutrients) within liquid environments on icy bodies represents a key uncertainty in the decision hierarchy for planetary protection. In future missions, observation techniques applied to different icy bodies can determine the concentration of these elements, as well as of compounds containing these elements. Further progress in understanding the chemistry of the early solar nebula from which the icy bodies accreted will also be important for constraining the abundances of key elements. An especially important research area for constraining the availability of key elements for terrestrial biological contaminants is the solubility of these elements and compounds under the conditions found within icy bodies. Theoretical modeling and laboratory analog studies will further constrain aqueous solubility and water-rock interactions under the pressures, temperatures, pH conditions, and solute conditions expected within icy bodies. Such studies are especially needed at the high pressures encountered within large icy bodies, because little is known about the possible interactions of rocks and brines with high-pressure ice phases.

Recommendation: **Research should be undertaken to determine the concentrations of key elements or compounds containing biologically important elements on icy bodies in the outer solar system through observational technologies and constraints placed on the range of trace elements available through theoretical modeling and laboratory analog studies.**

GLOBAL MATERIAL TRANSPORT

Understanding global chemical cycles and global material transport on icy bodies is important for several planetary protection decision points, notably the availability of elements, the availability of chemical energy sources, and the possibility of transport of spacecraft components on an icy body's surface into a subsurface liquid environment. The key to understanding this transport is to examine the geologic processes that can promote surface-subsurface exchange, to determine the rate at which they occur, the depth to which they penetrate, the influence of materials other than water ice mixed into the icy shells, and the role of liquid water in their operation. The concept of a "no-mans land" that bars transport of material into the subsurface also deserves closer scrutiny. When examined through this lens, and in combination with observed surface geology, several icy bodies may fall into the category of no concern. Further spacecraft exploration and reconnaissance of icy body geology and surface characteristics will continue to improve understanding of the global material transport cycles of icy bodies.

Recommendation: **Research should be undertaken to understand global chemical cycles within icy bodies and the geologic processes occurring on these bodies that promote or inhibit surface-subsurface exchange of material.**

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Appendixes

A
Letter Requesting this Study

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

MAY 2 0 2010

Reply to Attn of: SMD/Planetary Science Division

Dr. Charles F. Kennel
Chair
Space Studies Board
National Research Council
500 Fifth Street, NW
Washington, DC 20001

Dear Dr. Kennel:

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

In 2000, the SSB published a report entitled Preventing the Forward Contamination of Europa that provided advice regarding approaches for avoiding contamination by Earth life of subsurface oceans on Europa. Interest in exploring Europa and other icy bodies in the outer solar system has increased within both NASA and the international space exploration community, stimulated by data collected from current missions, as well as the recognition that international collaborative missions have the potential to provide scientific returns significantly greater than is possible with missions by individual space agencies. As NASA prepares for these future collaborative missions, it would be very helpful for the SSB to review the findings of the 2000 Europa report and incorporate conclusions from a series of recent workshops on planetary protection for icy bodies sponsored by COSPAR, in which it was determined that the probabilistic approach for regulating contamination of icy bodies should be retained to accommodate the wide range of objects for which requirements must be set. Ideally, this study would update and expand previous SSB recommendations to cover, as much as is currently feasible, the entire range of icy bodies in the outer solar system (asteroids, satellites, Kuiper-Belt Objects, comets) in light of current scientific understanding and ongoing improvements in mission-enabling capabilities and technologies.

Specifically, the SSB would consider the following subjects and make recommendations, as appropriate, in a report to NASA:

- The possible factors that usefully could be included in a Coleman-Sagan formulation describing the probability that various types of missions might contaminate with Earth life any liquid water, either naturally occurring or induced by human activities, on or within specific target icy bodies or classes of objects;
- The range of values that can be estimated for the above factors based on current knowledge, as well as an assessment of conservative values for other specific factors that might be provided to missions targeting individual bodies or classes of objects; and
- Scientific investigations that could reduce the uncertainty in the above estimates and assessments, as well as technology developments that would facilitate implementation of planetary protection requirements and/or reduce the overall probability of contamination.

In order for NASA to present the results of this study activity to the COSPAR Panel on Planetary Protection at the 2012 Colloquium, and to include the recommendations during development of joint ESA-NASA Europa-Jupiter System Mission concepts, it would be highly desirable to receive a final report by January 2012.

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

Sincerely,

Edward J. Weiler
Associate Administrator for
Science Mission Directorate

B
Current and Prospective Missions to Icy Bodies of Astrobiological Interest

For most of the history of the planetary program, the outer solar system has been accessible only by flagship missions characterized by large multidisciplinary payloads, relatively high costs (typically over $2 billion), and a requirement for high reliability. Such large missions—e.g., Galileo and Cassini—could be flown infrequently (approximately one per decade), and many new technologies and techniques were developed to support these projects.

As budget pressures on the space science program have increased, NASA and the science community have been motivated to find ways to enable outer solar system exploration within smaller, cost-constrained mission programs such as Discovery and New Frontiers. These missions are characterized by smaller spacecraft, highly focused science goals, lower costs, and more frequent launches. Flagship missions to the outer solar system continue to be a high priority both within the NASA strategic plan and in the planetary science decadal surveys,[1,2,3] but they will be cost-constrained and somewhat less ambitious than in the past and probably less frequent. This distinction between flagship and competed missions is important because competed missions are typically only selected approximately 3 to 4 years prior to launch. With such a short planning horizon, there is little time for NASA and the science community to debate and agree upon planetary protection requirements and standards on a mission-by-mission basis; thus, it is imperative that such standards be developed well in advance and that they are not unnecessarily burdensome or overly conservative.

As discussed in this report, the icy bodies of greatest potential concern from a planetary protection perspective are Europa, Enceladus, Titan, and Triton. All four are objects of high scientific priority for both astrobiological and non-astrobiological reasons. As such, it is instructive to review the plans for spacecraft missions to these objects, which have been developed in recent years, primarily by NASA and the European Space Agency (ESA). Exploration plans for other icy bodies—i.e., comets, Ganymede, the satellites of Uranus, other small satellites, Trojan asteroids, and Kuiper belt objects—are not discussed because these bodies are not expected to impose any significant planetary protection concerns. The missions described here should be considered as examples only, since none of them are currently funded or scheduled.

EUROPA

A Jupiter Europa Orbiter (JEO) was identified as a top science priority in the 2011 planetary science decadal survey (Box B.1).[4] It has been planned to launch in 2018-2020 as part of a joint NASA-ESA project known as the Europa Jupiter System Mission (EJSM), which would comprise both the Europa orbiter as well as an ESA-developed Ganymede orbiter. JEO would place a spacecraft equipped with remote sensing and radar investigations into a close orbit around Europa for a period of at least 1 year. Prior to insertion into Europa orbit, JEO would complete a 2-year tour of the jovian system using the Galilean satellites for gravity-assist flybys. Given the complex gravitational environments of the jovian system, the long-term stability of JEO's orbit about Europa cannot be guaranteed. Therefore, to meet planetary protection requirements at the end of its mission, JEO would be either commanded to impact onto the surface of Europa in a controlled manner at a selected site, or ejected from Europa orbit and placed on collision course with Jupiter. The combination of this controlled end-of-mission scenario, along with standard clean assembly procedures, selective application of dry-heat microbial reduction, and the sterilizing effect of the jovian radiation environment, would allow JEO to meet planetary protection requirements. The integrated cost of these requirements, while not a primary driver of the mission budget, is nonetheless significant.

As of this writing, budget pressures have led to a descoping and replanning of JEO and probably of the entire EJSM program. Current studies are focused on developing less costly JEO mission concepts. Once those studies are complete and the budget picture is clarified, NASA will decide whether and how to proceed with Europa exploration; in the meantime, ESA is continuing its studies of the Ganymede orbiter element of EJSM. When a Europa mission is flown, a key aspect of mission affordability will be adoption of the streamlined planetary protection decision framework recommended in this report.

ENCELADUS

The 2011 planetary science decadal survey also recommended that NASA consider studying a flagship mission to Enceladus (Box B.2).[5] The Enceladus Orbiter would investigate the satellite's cryovolcanic activity, habitability, internal structure, chemistry, geology, and interaction with other bodies within the saturnian system. As is the case for a Europa orbiter, the complex gravitational environments of the saturnian system implies that the long-term stability of an orbiter about Enceladus cannot be guaranteed. Thus, special measures would be needed to ensure that the ultimate fate of the Enceladus Orbiter is consistent with planetary protection provisions.

An Enceladus Orbiter mission was accorded a lower priority than either the Europa Orbiter or a proposed Uranus Orbiter and was recommended for flight only if those other two missions could not be accomplished for cost or technical reasons. Thus it is likely that a flagship Enceladus Orbiter will not take place until after 2025, and possibly not until the 2030s.

It should be noted that, given the high science priority of both Europa and Enceladus, it is possible, although far from certain, that a mid-term update to the decadal survey could include one or both of those targets in the list of candidates for future New Frontiers missions. That could provide an earlier pathway to flight for missions to those bodies, albeit with reduced costs and more constrained science goals. Thus, even in the absence of a clear plan for near-term flagship missions to Europa and Enceladus, it is important to be cognizant of their unique planetary protection requirements so that the community can be prepared to propose such missions should the opportunity arise.

TITAN

NASA and ESA have sponsored extensive studies of missions to Titan over the years, and a large multiplatform Titan Saturn System Mission (TSSM) was considered as a possible near-term flagship mission (Box B.3).[6,7] For reasons of cost and technology readiness, the recent planetary science decadal survey deferred that mission to the subsequent decade (after 2022). Titan science, however, remains a high priority due to the unique characteristics of the satellite's atmosphere and the discovery of hydrocarbon lakes on its surface. Titan is believed to represent an environment in which prebiotic chemical processes, similar to those that were active on early Earth, can be studied in depth.

Within the low-cost Discovery program, NASA is currently evaluating the so-called Titan Mare Explorer (TiME) as a candidate for launch in 2016.This would be a highly focused investigation of the composition and characteristics of a northern hemisphere Titan sea using a floating platform. The decision on whether to fly this mission or one of the other two candidates will be made in mid-2012. Titan may also be considered as a potential New Frontiers candidate during a midterm update to the planetary science decadal survey.

As discussed previously in this report, planetary protection is not a major consideration for missions to Titan because of the cryogenic temperatures, limited or no access to liquid water, and lack of phosphorus to support cell growth. Standard clean assembly procedures and bioburden assays are expected to be sufficient for all future Titan missions. It is important to note that Titan missions with a strong focus on prebiotic chemistry will likely face rigorous constraints on organic cleanliness analogous to those placed on the biological cleanliness of missions carrying life-detection experiments.

TRITON

Missions to Neptune and its large satellite, Triton, have been identified in prior NASA strategic plans as high priorities for the long term. Like the TSSM, a Neptune Orbiter and Probe mission was identified in the recent planetary science decadal survey as a high science priority. For reasons of cost and technology readiness, however, it was not recommended for development in the coming decade.[8] A dedicated Triton mission was not included in the decadal survey recommendations, although it is anticipated that a flagship Neptune Orbiter would also conduct extensive Triton science. Planetary protection planning for Triton would thus focus on ensuring that a Neptune Orbiter was developed with appropriate safeguards, including standard clean assembly, bioburden assays, and selective dry-heat microbial reduction.

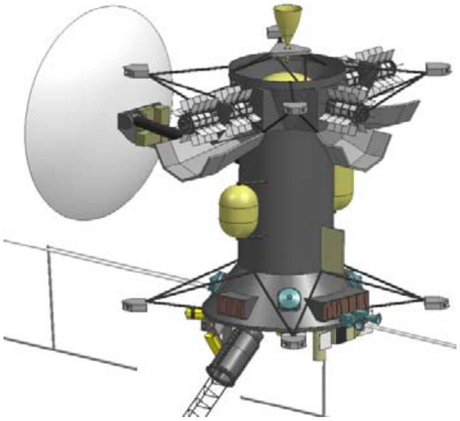
As with the Europa, Enceladus, and Titan, it is possible that future Discovery or New Frontiers missions may propose investigation of the Neptune/Triton system, and these may represent earlier launch opportunities than would be possible within a flagship mission paradigm. Such a mission to the Neptune/Triton system would be very challenging within the current cost caps and would likely be enabled by new technologies that are only now under study. Thus it is expected that Triton missions are far enough in the future as to not be appropriate drivers for specific planetary protection recommendations at this time.

MISSIONS TO OTHER ICY BODIES

The outer solar system is home to a large number of icy bodies that are scientifically interesting for reasons other than astrobiology. These include comets, Trojan asteroids, trans-Neptunian objects, and the small satellites of Uranus and the other giant planets. It is generally expected that missions to these bodies will undergo standard clean assembly procedures as are followed in all planetary missions but will not be required to meet any other planetary protection requirements due to their lack of liquid water, sources of energy, and/or chemical constituents that can promote cell growth. Thus eventual missions to these targets will be governed under the decision rules contained in this report and should impose no unique requirements.

BOX B.1 Jupiter Europa Orbiter

Jupiter Europa Orbiter



SOURCE: NASA Mission Study (transmitted from Curt Niebur, NASA SMD/Planetary Science Division)

Scientific Objectives

- **Explore Europa to investigate its habitability**
- **Key science issues addressed:**
  - *Characterize the extent of the europan ocean and its relation to the deeper interior*
  - *Characterize the ice shell and any subsurface water including the nature of the surface-ice-ocean exchange*
  - *Determine global surface compositions and chemistry, especially related to habitability*
  - *Understand the formation of surface geology, including sites of recent or current activity and characterize sites for future in situ exploration*
  - *Understand Europa in the context of the Jupiter system*

Key Challenges

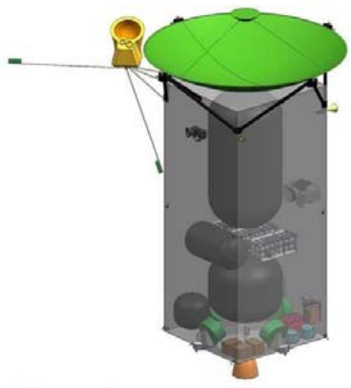
- **Radiation**
  - *Systems engineering for electronics vault repartitioning*
  - *"Fail operational" fault management to handle environment*
- **Mass**
  - *Uncertainty in instrument and shielding mass*
  - *Low launch margin for this development phase*
  - *Overall sensitivity of system mass to changes*
- **Power**
  - *System impacts of changing number and design of radioisotope power system units*
  - *Availability of plutonium-238*
- **Instruments**
  - *Uncertainties in design of model payload*

Key Parameters

- **Model Payload**
  - *Ocean: Laser Altimeter, Radio Science*
  - *Ice: Ice Penetrating Radar*
  - *Chemistry: Vis-IR Imaging Spectrometer, UV Spectrometer, Ion and Neutral Mass Spectrometer*
  - *Geology: Thermal Instrument, Narrow Angle Imager, Wide and Medium Angle Imager*
  - *Particles and Fields: Magnetometer, Particle and Plasma Instrument*
- **Five Multi-Mission Radioisotope Thermal Generators**
- **Launch Mass: 4745 kg**
- **Launch Date: 2020 (on Atlas V 551)**
- **Orbit: 100-200 km Europa orbit + jovian tour**

Box B.2 Enceladus Orbiter

Enceladus Orbiter Spacecraft



SOURCE: NASA Mission Study (transmitted from Curt Niebur, NASA SMD/Planetary Science Division)

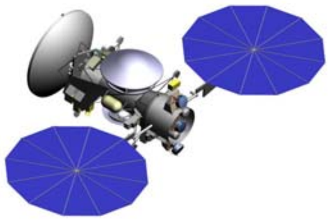
Key Challenges
- **Planetary Protection**
  - *Potential modifications to design required if planned Enceladus impact disposal is not acceptable for Planetary Protection*
- **Particle Impact Damage**
  - *Potential for spacecraft damage from Saturn E-ring or Enceladus plume particle impact*
  - *Primary concern is High Gain Antenna surface quality*
- **System Power**
  - *Some potential for reduced science operations with assumed ASRG degradation*

Scientific Objectives
- **Investigate the internal structure, geology, and chemistry of Enceladus and plumes discovered by Cassini**
- **Prepare for potential future landing**
- **Observe interactions between Enceladus and the Saturn system and explore the surfaces and interiors of Saturn's moons**
- **Key science issues addressed:**
  - *Investigate the nature of Enceladus's cryovolcanic activity*
  - *Provide improved measurements of plume gas and dust*
  - *Measure tidal flexing, magnetic induction, static gravity, topography, and heat flow*

Key Parameters
- **Payload**
  - *Medium Angle Imager*
  - *Thermal Imaging Radiometer*
  - *Mass Spectrometer*
  - *Dust Analyzer*
  - *Magnetometer*
- **Three Advanced Stirling Radioisotope Generators**
- **Launch Mass: 3560 kg**
- **Launch Date: 2023 (on Atlas V 521)**
- **Orbit: Enceladus orbit (100 km x 267 km, 62 deg inclination) plus Saturn satellite tour**

Box B.3 Titan Saturn System Mission

Titan Orbiter + Balloon and Lake Lander



SOURCE: NASA Mission Study (transmitted from Curt Niebur, NASA SMD/Planetary Science Division)

Key Challenges

- **In Situ ESA-supplied Elements**
  - *Uncertainty in accommodation, pending element maturation*
  - *Element operations and communications relay using Orbiter*
- **Mass**
  - *Uncertainty in instrument mass*
  - *Low launch margin for this development phase*
- **Power**
  - *Battery recharge time in Titan orbit*
  - *Impact of switching to MMRTGs from ASRGs*

Scientific Objectives

- **Explore Titan as an Earth-like system**
- **Examine the organic chemistry of Titan's atmosphere**
- **Explore Enceladus and Saturn's magnetosphere for clues to Titan's origin and evolution**
- **Key science issues addressed:**
  - *Exploring organic-rich environments*
  - *Origin and evolution of satellite systems*
  - *Understanding dynamic planetary processes*

Key Parameters

- **Model Payload**
  - *High Resolution Imager and Spectrometer*
  - *Titan Penetrating Radar and Altimeter*
  - *Polymer Mass Spectrometer, Sub-Millimeter Spectrometer, Thermal Infrared Spectrometer*
  - *Magnetometer, Energetic Particle Spectrometer, Langmuir Probe, Plasma Spectrometer*
  - *Radio Science and Accelerometers*
- **In Situ Elements: Balloon and Lake Lander**
- **Radioisotope Power Sources: 5 ASRGs + 1 MMRTG**
- **Launch Mass: 6203 kg**
- **Launch Date: 2020 (on Atlas V 551) gravity assist SEP**
- **Orbit: 1500 km Titan orbit + Saturn tour including Enceladus flybys**

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C
Event Sequence Diagram for the Determination of Planetary Protection Measures for Missions to Icy Bodies

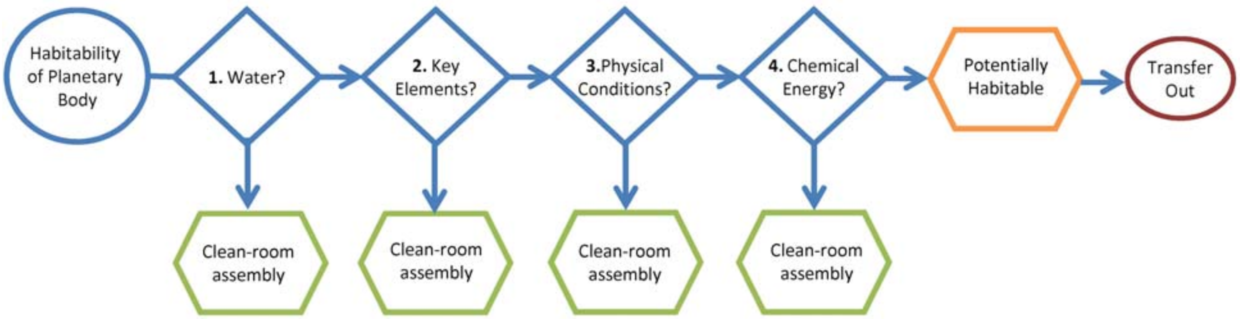
The binary decision-making framework outlined in Chapter 2 provides an alternative to probabilistic estimates of contamination constrained by the uncertain and/or unknowable factors included in the Coleman-Sagan equation. The decision-making framework can be visualized in a number of different ways. The committee's preferred depiction (see Figure 2.2) may not be the one most familiar to all relevant scientific and technical communities. Indeed, engineers tend to visualize decision networks as event sequence diagrams.

The event sequence diagram presented in Figure C.1 is included to provide mission planners with the functionally equivalent of the decision-making framework in Chapter 2, but in a more familiar format.

Figure C.1 indicates the process to be applied for the two determinations necessary, the first of which is related to potential habitability of the icy body target (that is, its "fragility" against bio-propagation), and the second related to the type of mission proposed so as to address the potential for "initiating" a bio-contamination of a potentially habitable icy body. This bimodal determination process (that is, the determination of the fragility of the process, design, target) and the determination of the potential for damage initiation is consistent with the general process of risk determination used across a variety of applications.[1,2]

The left-hand portion of Figure C.1 represents the decision of whether the planetary body of interest should be considered to be potentially habitable. Four criteria are used to judge the habitability of the planetary body and specifically question whether the planetary body is known to possess liquid water, the key elements considered essential for terrestrial life, environments known to be compatible with known extreme conditions of terrestrial life, and accessible sources of chemical energy. If the planetary body does not possess one or more of these attributes, then it is judged as inhabitable to terrestrial life and, while assembly of spacecraft intended for these bodies should be performed in a cleanroom, no bioload reduction is required for planetary protection. If the planetary body does possess these four essential attributes for habitability of terrestrial life, or if this information remains undetermined at the time of the mission, then it is deemed to be potentially habitable.

The right-hand portion of Figure C.1 considers the nature of the mission itself (e.g., flyby, orbiter, lander) as relevant to determining planetary protection requirements for missions to potentially habitable planetary bodies. Consideration must be given to whether the mission employs a lander and/or an orbiter and whether a flyby attempt will be made of the given planetary body. If a lander is employed, the likelihood of the spacecraft interacting with a habitable region must be evaluated, and for all missions the probability of the lander crashing or otherwise interacting with a region where surface—subsurface transport is possible must be assessed. If this likelihood is less than 10^{-4} over a period of 10^{3} years, then no bio-load reduction measures are required for planetary protection beyond cleanroom assembly. If the probability for interacting with habitable regions exceeds 10^{-4} over a period of 10^{3} years, then specific consideration must be given to whether the lack of complex and heterogeneous organic nutrients in aqueous environments of icy moons would preclude the propagation of any microbes that may have survived extreme irradiation and desiccation environments in transport. If the lack of nutrients indeed precludes propagation, then clean-room assembly is deemed sufficient; however, if the potential for propagation remains, then at least minimal planetary protection methods are required, and the final decision question then considers whether heat treatment at 60°C for 5 hours would fail to eliminate all physiological groups that can potentially propagate on the target body. If so, then stringent planetary protection methods are required for the mission to proceed, or else the mission must either be reformulated or cancelled.

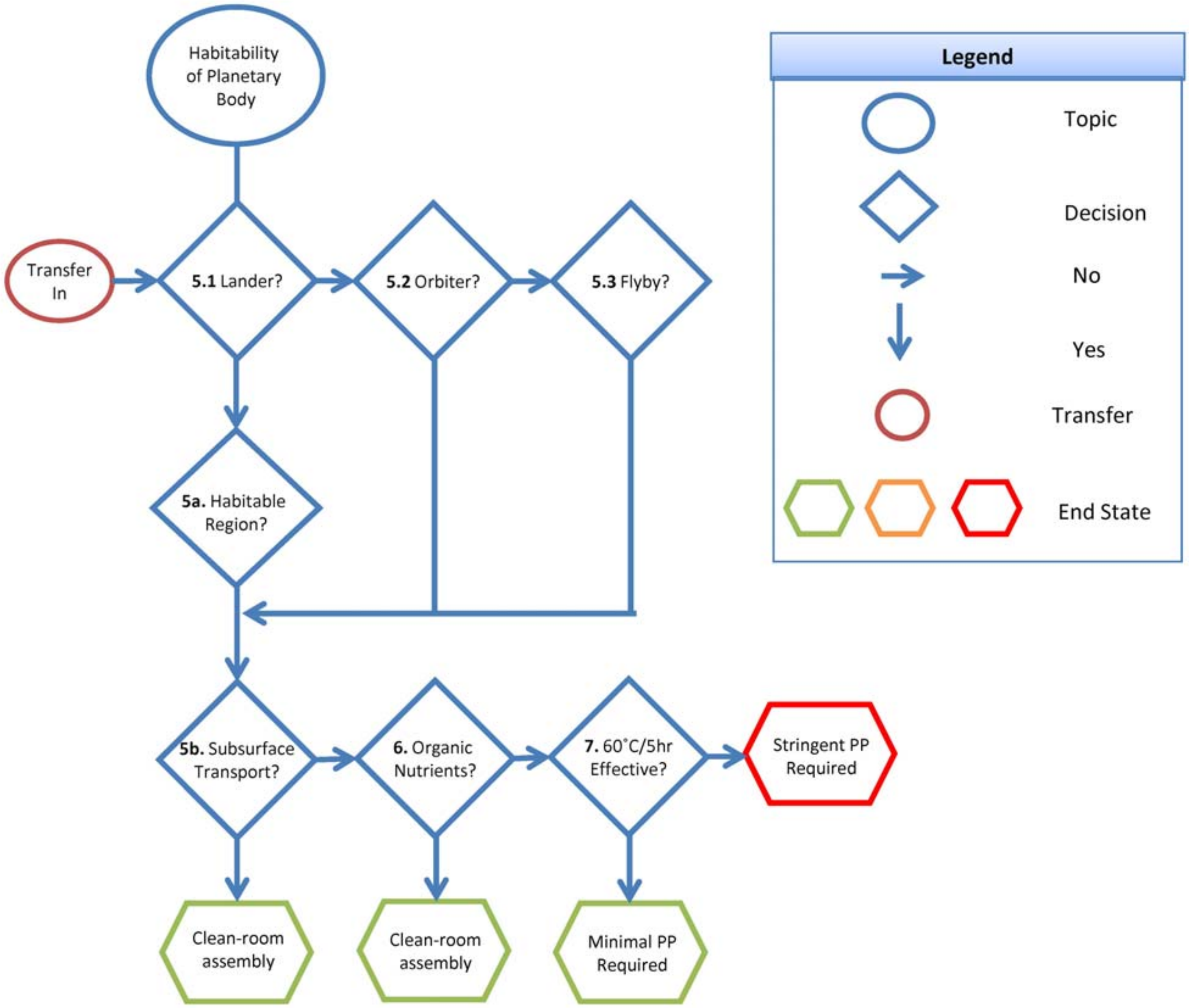


Key to Decision Questions

1. Do current data indicate that the destination lacks liquid water essential for terrestrial life? (*Decision Point 1*)
2. Do current data indicate that the destination lacks any of the key elements C, H, N, P, S, K, Mg, Ca, O, and Fe, required for terrestrial life? (*Decision Point 2*)
3. Do current data indicate that the physical properties of the target body are incompatible with known extreme conditions for terrestrial life? (*Decision Point 3*)
4. Do current data indicate that the environment lacks an accessible source of chemical energy? (*Decision Point 4*)

5.1. Is a lander available? (*Decision Point 5*)
5.2. Is an orbiter available? (*Decision Point 5*)
5.3. Is a close flyby possible? (*Decision Point 5*)
5a. Do current data indicate that the probability of the spacecraft contacting a habitable environment within 1,000 years is less than 10^{-4}? (*Decision Point 5*)
5b. Do current data indicate that the probability of the spacecraft crashing or otherwise contacting an active fissure or other region where surface-subsurface transport is possible within 1,000 years is less than 10^{-4}? (*Decision Point 5*)

6. Do current data indicate that the lack of complex and heterogeneous organic nutrients in aqueous environments of icy moons will prevent the survival of irradiated and desiccated microbes? (*Decision Point 6*)
7. Do current data indicate that heat treatment of the spacecraft at 60°C for 5 hours will eliminate all physiological groups that can propagate on the target body? (*Decision Point 7*)



Key to Planetary Protection Endpoints

Clean-room assembly	Clean Room assembly but no bio-load reduction required for Planetary Protection
Minimal PP Required	Minimal Planetary Protection required including NASA standard cleaning and bioload monitoring, heating sealed components to 60˚C for 5 hours and molecular bioload analysis
Stringent PP Required	Stringent Planetary Protection required, including NASA standard cleaning and bioload monitoring, molecular bioload analysis, and Viking-level terminal bioload reduction OR decline mission

REFERENCES

1. J. Fragola, B. Putney, and J. Minarck III, *An Evaluation of Containment Assurance Risk for Earth Entry Vehicle and Space Shuttle Sample Return,* Earth Entry Vehicle Office, NASA Langley Research Center Hampton, Va., September 30, 2002.

2. J. Fragola, B. Putney, and J. Minarck III, *Mars Sample Return Probabilistic Risk Assessment Final Report: An Evaluation of Containment Assurance Risk for Earth Entry Vehicle and Space Shuttle Sample Return,* Contract No. 123-4119, NASA Langley Research Center, Hampton, Va.

D
Committee and Staff Biographical Information

MITCHELL L. SOGIN, Chair, is a senior scientist and director of the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution at the Marine Biological Laboratory (MBL) in Woods Hole, MA. He also is a professor in the Department of Molecular Biology, Cell Biology and Biochemistry for the Brown University—MBL Graduate Program in Biological and Environmental Sciences. Prior to joining MBL in 1989, he was on the faculty of the University of Colorado Health Sciences Center. His current research focuses on molecular evolution of protists, microbial diversity in extreme environments, and microbial oceanography. He is an elected Fellow of the American Academy of Microbiology, the American Academy of Arts and Sciences, and the AAAS, and he currently serves on the editorial boards of four scientific journals. He received his Ph.D. in microbiology and molecular biology from the University of Illinois at Urbana-Champaign. His past NRC service includes membership on the Space Studies Board, Committee on the Limits of Organic Life in Planetary Systems, Committee on Preventing the Forward Contamination of Mars, Committee on a New Science Strategy for Solar System Exploration, Steering Group for the Workshop on Size Limits of Very Small Microorganisms, and Task Group on Sample Return from Small Solar System Bodies.

GEOFFREY COLLINS, Vice Chair, is associate professor of geology at Wheaton College. He joined the faculty at Wheaton College in 2000 after receiving his Ph.D. in geological science from Brown University. He is a planetary scientist who is interested in geologic processes on the icy satellites of the outer solar system, including Ganymede and Europa at Jupiter and Enceladus, Dione, and Titan at Saturn.

AMY BAKER is the owner of Technical Administrative Services (TAS), a technically based service organization that provides technical and administrative support focusing specifically on the needs of the international scientific community. Over the past 6 years under TAS auspices she has participated in research for new biological methods for inclusion in NASA's planetary protection procedures. As senior engineer with Lockheed Martin Astronautics, Ms. Baker worked as the Technical Lead for the Planetary Protection Laboratory for the Mars Surveyor Program and the Chemical Technology Laboratory. During her tenure with the National Renewable Energy Laboratory she served as the Deputy Director for the Hydrogen Research Program for the Department of Energy. Ms. Baker was also the Secretariat to the International Energy Agency Executive Committee on Hydrogen Research. She was a member of the NRC Committee on Principles of Environmental and Scientific Stewardship for the Exploration and Study of Subglacial Lake Environments.

JOHN A. BAROSS is professor of Oceanography and the Astrobiology Program at the University of Washington, Seattle. His research specialty is the ecology, physiology and molecular phylogeny of microorganisms and viruses from hydrothermal vent and subseafloor environments. Dr. Baross has particular interests in the microbiology of extreme environments and in the significance of submarine hydrothermal vent systems for the origin and evolution of life and for the possibility of life on other planets in similar settings. He received his Ph.D. in marine microbiology from the University of Washington. He is a fellow of the American Academy of Microbiology and an national associate of the National Research Council. His NRC service includes chairing the Committee on the Limits of Organic Life in Planetary Systems, co-chairing the Committees on the Origins and Evolution of Life and for a Review of Programs to Determine the Extent of Life in the Universe, and membership on the Steering

Group for the Workshop on Size Limits of Very Small Microorganisms, the Task Group on Sample Return from Small Solar System Bodies, and the Ad Hoc Task Group on Mars Planetary Protection.

AMY C. BARR is an assistant professor in the Department of Geological Sciences at Brown University. Her research interests are in planetary mantle convection with emphasis on icy satellites, planetary impacts, ice physics, and nonlinear fluid dynamics. After completing her PhD at the University of Colorado in 2004, she held a post-doctoral position at Washington University, St. Louis, and then joined Southwest Research Institute, where she remained until 2011. She has been a principal investigator in the NASA Cassini data analysis program, outer planets research program, and planetary geology and geophysics program. Her current research interests are in accretion and early thermal evolution of planetary satellites; planetary impacts; heat transfer in solid planets; evolution of grain size and crystal fabric in ice; and the relationship between solid-state convection and resurfacing on icy bodies. Dr. Barr is a graduate of the California Institute of Technology where she completed a B.S. in Planetary Science. In 2007 Dr. Barr received a NASA early career fellowship.

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

CHARLES S. COCKELL is a professor of Astrobiology at the School of Physics and Astronomy at the Edinburgh University. He was formerly a professor and chair of geomicrobiology in the Center for Earth, Planetary, Space, and Astronomical Research (CEPSAR), at the Open University in the United Kingdom. His research focuses on microbe-mineral interactions and the way in which microorganisms colonize and live in rocky environments in the solar system. His committee service has included membership on the ESA Life Sciences Working Group, the ESA Planetary Protection Working Group, the ESA Science and Technology Advisory Group, and the NASA Mars Human Precursor Science Steering Group. He is a Fellow of the Royal Geographical Society, the Explorers Club of New York, the British Interplanetary Society, and the Royal Astronomical Society. He has authored two popular science books and co-editor of four scientific books. In 1994 he founded the Earth and Space Foundation, a charity which awards grants to expeditions that successfully bridge the gap between environmentalism and the exploration and settlement of space by either using space technologies and ideas in environmental fieldwork or using environments on Earth to advance knowledge of other planets. He received his D.Phil. in Molecular biophysics from the University of Oxford.

MICHAEL J. DALY is a Professor of Pathology at the School of Medicine at the Uniformed Services University of the Health Sciences. He is an expert in the study of bacteria belonging to the family Deinococcaceae, which are some of the most radiation-resistant organisms yet discovered. His NRC service includes membership on the Committee on Planetary Protection Requirements for Venus Missions, Committee on the Origins and Evolution of Life, Committee on the Astrophysical Context of Life, and Task Group on the Forward Contamination of Europa.

JOSEPH R. FRAGOLA is vice president of Valador, Inc., a small information architecture consulting firm. Mr. Fragola has more than 35 years of experience working in reliability and risk technology in both the aerospace and nuclear industries. He is a professional engineer and received his B.S. and M.S. degrees in physics from the Polytechnic Institute of New York. In the past he has worked for Grumman Aerospace Corporation and for IEEE at their headquarters in New York. He was recently a principal scientist at SAIC and continues to be a visiting professor at the University of Strathclyde in Glasgow, Scotland. He has published nearly 50 papers and two books. He was awarded the P.K. McElroy RAMS Best Paper Award in 1993, the Alan Plait Award for the Best Tutorial in 2004, and the IEEE Region I Award and has been named an IEEE fellow for his contributions to the theory and practice of risk, safety, and reliability.

ROSALY M.C. LOPES is a senior research scientist at the Jet Propulsion Laboratory and Deputy Manager for the Planetary Sciences section. Her research interests focus on use of remote sensing data collected from spacecraft to further develop theoretical models of surface processes, including analysis of Io's infrared spectra obtained by Galileo's Near-Infrared Mapping Spectrometer and analysis of geologic features on Titan using the Cassini Radar Mapper, with particular emphasis on cryovolcanic features. Recognitions for her work include Wings WorldQuest: Women of Discovery Award, Fellow of the AAAS, Carl Sagan Medal from the American Astronomical Society's Division for Planetary Sciences, and Latinas in Science Award from the Comision Feminil Mexicana Nacional. She is the author or co-author of five books and nearly 100 peer-reviewed publications. She served as a member of the NRC Committee on New Opportunities in Solar System Exploration.

KENNETH H. NEALSON is the Wrigley Professor of Geobiology at the University of Southern California. His research interests focus on environmental microbiology and biogeochemistry. Dr. Nealson previously served as a senior scientist at NASA's Jet Propulsion Laboratory and as a professor at the Center for Great Lakes Studies of the University of Wisconsin, Milwaukee. He is a fellow of the American Academy of Microbiology, recipient of the Distinguished Visiting Researcher Award from the Joint Oceanographic Institution, and recipient of the Cecil and Ida Green Visiting Professorship at the Carnegie Institution of Washington. In addition, he received the 2003 Proctor and Gamble Award in Applied and Environmental Microbiology. He received his Ph.D. in Microbiology from the University of Chicago. Dr. Nealson has served on the NRC's Space Studies Board, the Task Group on Issues in Sample Return, the Ad Hoc Task Group on Planetary Protection, the Committee on Planetary Biology and Chemical Evolution, and the Committee on the Origins and Evolution of Life.

DOUGLAS S. STETSON is a consultant, specializing in innovative mission and system concepts, strategic planning, decision analysis, proposal development, and university and industry partnerships. He is the founder and president of the Space Science and Exploration Consulting Group, a network of senior advisors and experienced individuals drawn from NASA, national laboratories, industry, and universities. Prior to becoming a consultant, Stetson spent 25 years at the Jet Propulsion Laboratory in a variety of technical and management positions, including several assignments at NASA Headquarters. At JPL he was most recently the manager of the Solar System Mission Formulation Office, responsible for development of all new planetary mission and technology strategies and programs. Earlier in his career, he played key roles in the design and development of several major planetary missions, including Cassini and Galileo, and was the leader of many planetary advanced studies and proposals. He was a member of the Inner Planets Panel for the NRC Planetary Science Decadal Survey.

MARK H. THIEMENS is Dean of Physical Sciences, Distinguished Professor of Chemistry, and Chancellor's Associates Chair in the Department of Chemistry and Biochemistry at the University of California, San Diego. His research is centered on use of the mass-independent fractionation process for stable isotopes to study the origin and evolution of the solar system, definition of the source and transformation of greenhouse gases in the troposphere, chemistry of the stratosphere and mesosphere, chemistry of the ancient Martian atmosphere, and the origin and evolution of oxygen-ozone and life in the Earth's Precambrian. Dr. Thiemens is a member of the National Academy of Sciences (NAS) and his extensive NAS-NRC service includes membership on the Board on Energy and Environmental Systems, PNAS Editorial Board, and Committee on the Review of Planetary Protection Requirements for Mars Sample-Return Missions.

Staff

DAVID H. SMITH joined the Space Studies Board in 1991. He is the senior staff officer and study director for a variety of NRC activities in planetary science, astrobiology, and astrophysics. He also organizes the SSB's Lloyd V. Berkner Summer Policy Internship program and supervises most, if not all, of the interns. He received a B.Sc. in mathematical physics from the University of Liverpool in 1976, completed Part III of the Mathematics Tripos at Cambridge University in 1977, and earned a D.Phil. in theoretical astrophysics from Sussex University in 1981. Following a postdoctoral fellowship at Queen Mary College University of London (1980-1982), he held the position of associate editor and, later, technical editor of *Sky and Telescope*. Immediately prior to joining the staff of the SSB, Dr. Smith was a Knight Science Journalism Fellow at the Massachusetts Institute of Technology.

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

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

HEATHER D. SMITH recently completed her PhD in biological engineering from Utah State University. After earning a bachelor's degree in psychology from the University of North Texas, Dr. Smith moved to California to work as a Space Camp counselor. While at Space Camp she volunteered at NASA Ames Research Center and was hired as a research associate for The SETI Institute upon completion of an undergraduate degree in physics from The Evergreen State College. After working at Ames for several years she decided to go back to graduate school. Prior to her doctoral degree she earned an MSc in space studies from International Space University.

ANNA B. WILLIAMS received her Ph.D. in organic chemistry from Northeastern University. Her doctoral research focused on the development of small organic mimics of the protein alpha helix, for use as inhibitors of protein-protein interactions. Another aspect of her work was in the development of synthetic methodology towards the efficient radiolabeling of compounds of known biological activity for use as radioactive tracers. Prior to her graduate work, Dr. Williams received her bachelor's degree in chemistry with a minor in philosophy from Dickinson College.

KATIE DAUD is a senior at Bloomsburg University of Pennsylvania with a triple major in planetary science, Earth science and political science. She serves as the president of the Astronomy Club, Senator for the Community Government Association and Chair of the Student Organizations Committee. She did research for the Smithsonian National Air and Space Museum on lunar tectonics. She is interested in combining both her passion for space exploration and her skills in policy to work for NASA's Office of Legislative and Intergovernmental Affairs.

DANIELLE PISKORZ grew up on Long Island, New York, and recently graduated from the Massachusetts Institute of Technology with a degree in physics and a minor in applied international studies. She has done various research projects at L'Institut d'Astrophysique de Paris, Los Alamos National Laboratories, and Jet Propulsion Laboratory and spent her junior year studying at the University of Cambridge. Ms. Piskorz plans to begin her graduate studies at the California Institute of Technology in the Fall of 2012. In the meantime, she intends to gain meaningful experience in science policy with the hope of making a contribution to the field in the future.

E
Glossary and Abbreviations

Aeolian—Geologic processes involving wind.

Archaea—Organisms making up one of the three branches on the phylogenetic tree of life. Their cells do not contain a defined nucleus and they are genetically and biochemically distinct from the Bacteria. See **Eukaryotes** and **Bacteria.**

Astrobiology—The study of the origin, evolution and distribution of life in the universe.

Autotroph—Organisms than can use carbon dioxide as their sole source of carbon. See **Heterotroph**.

Bacteria—Organisms making up one of the three branches of the phylogenetic tree of life. Their cells do not contain a defined nucleus and they are genetically and biochemically distinct from the Archaea. See **Eukaryotes** and **Archaea.**

Centaurs—A family of small solar system bodies found between the orbits of Jupiter and Neptune, having appearances ranging from asteroidal to cometlike. Their orbital characteristics indicate that they have not resided in their present locations very long, leading to the suggestion that they are recently migrated Kuiper belt objects.

Chemoautotroph—Organisms with the ability to synthesize organic nutrients directly from simple inorganic compounds using the energy derived from chemical rather than photochemical reactions.

Chemolithoautotroph—Organisms deriving all of their carbon and energy requirements from inorganic compounds. The "litho" component of the name implies that they derive energy from the oxidation of hydrogen.

Clathrates—A compound in which one component is enclosed by the structure of another.

Coleman-Sagan—A methodology used to calculate the probability that terrestrial organisms on or within a spacecraft could survive and proliferate while transiting through space to an extraterrestrial planetary environment.

Commensurability—A location (e.g., in the asteroid belt) where a body orbits with a period that is a simple fraction (e.g., 2/5 or 1/3) of the period of another large body (e.g., Jupiter) where resonant effects can build up.

Containment—Physical and biological isolation and handling of returned samples as specified for samples returned from Mars.

COSPAR—(Committee on Space Research) intermediate body responsible for determining planetary protection requirements.

COSPAR categories—Categories, I-V, are a series of rules and requirements that have to be met depending on the object visited and the type of mission.

Category I—Includes any mission to a target body which is not of direct interest for understanding the process of chemical evolution or the origin of life. No protection of such bodies is warranted and no planetary protection requirements are imposed by this policy.

Category II—All types of missions to those target bodies where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote chance that contamination carried by a spacecraft could jeopardize future exploration. The requirements are for simple documentation only. Preparation is required for these flight projects primarily to outline intended or potential impact targets, brief Pre- and Post-launch analyses detailing impact strategies, and a Post-encounter and End-of-Mission Report which will provide the location of impact if such an event occurs.

Category III & IV —Certain types of missions to a target body of chemical evolution and/or origin of life interest or for which scientific opinion provides a significant chance of contamination which could jeopardize future biological experiments. Category III, mostly flyby and orbiter. Requirements will consist of documentation (more involved than Category II) and some implementing procedures, including trajectory biasing, the use of cleanrooms during spacecraft assembly and testing, and possibly bioburden reduction. Category IV, mostly probe and lander. Requirements imposed include rather detailed documentation (more involved than Category III), including a bioassay to enumerate the bioburden, a probability of contamination analysis, an inventory of the bulk constituent organics and an increased number of implementing procedures.

Category V—All Earth-return missions. The concern for these missions is the protection of the terrestrial system, Earth, and the Moon. For solar system bodies deemed by scientific opinion to have no indigenous life forms, a subcategory "unrestricted Earth return" is defined. For all other Category V missions, in a subcategory defined as "restricted Earth return," the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth.

Cryovolcanism—A low temperature analog of silicate volcanism where a volatile, such as water, ammonia, and methane plays the role of lava on the surface of a planetary body.

Diapirs—A dome or anticlinal fold in which a mobile plastic core has ruptured the more brittle overlying rock.

DNA (deoxyribonucleic acid)—A polymer of nucleotides connected via a sugar-phosphate backbone. This complex biomolecule encodes genetic information in all terrestrial organisms.

Downwelling—The downward movement of material of a body driven by buoyancy forces as in the case by a high density fluid sinking beneath a low density fluid.

Ejecta—Material that is thrown up from an event, such as an impact.

EJSM—Europa Jupiter System Mission.

Endogenic—Relating to a geologic process of internal origin (volcanism, tectonism).

ESA—European Space Agency.

Eukaryotes—Organisms making up one of the three branches on the phylogenetic tree of life. Their characteristic feature is that their cells have a defined nucleus containing most of the organism's DNA. See **Archaea** and **Bacteria.**

Extremophiles—Microorganisms capable of growing under extreme physicochemical conditions such as high temperatures, pressures, and acidity.

Facultative anaerobe—An organism with the capacity to grow in both the presence and the absence of oxygen. See **Aerobe** and **Anaerobe**.

Fluvial—Pertaining to or produced by the action of a river or stream.

Forward contamination—The biological contamination of an extraterrestrial body by terrestrial organisms inadvertently carried aboard a spacecraft.

Gram-negative bacteria—Bacteria that show a red color from Gram's stain procedure.

Gram-positive bacteria—Bacteria that shows a purple color from Gram's stain procedure. The structure of the bacterias' cell wall determines its ability to retain the dye used in the Gram-stain procedure.

Gray—A measure of radiation exposure defined in terms of the total amount of energy absorbed per unit mass of the absorbing material. One gray is equal to 1 joule of energy deposition per kilogram of the target material. Because the amount of energy absorbed depends on the nature of the target material, the unit is often qualified to indicate the nature of the target. One gray is equal to 100 rad.

Habitable zone—The notional region around a star within which an Earth-like planet would experience environmental conditions compatible with life as we know it. The solar system's habitable zone stretches, approximately, from the orbit of Venus to the orbit of Mars.

Heterotroph—An organism that survives by the ingestion and breakdown of complex organic materials. See **Autotroph**.

Hydrothermal vents—Springs of hot seawater on the deep ocean floor. They are formed when cold seawater seeps through cracks in the ocean floor, circulates through volcanically heated rock, and returns to the seafloor rich in dissolved minerals.

Hyperthermophiles—An organism adapted to living in high temperatures of 80°C or higher.

Impact gardening—The process by which the surface of atmosphere-less bodies are stirred and resurfaced by impacts.

JEO—Jupiter Europa Orbiter.

JPL—Jet Propulsion Laboratory.

Kuiper belt—A torus-shaped volume beyond the orbit of Neptune populated by bodies ranging up to many hundreds of kilometers in size; the source region for most short-period comets.

KBO's—Kuiper belt objects.

Lacustrine—Relating to lakes.

Magnetosphere—The volume of space surrounding a planetary body that is under the dynamical influence of that body's magnetic field.

Mesophilic—Preferring moderate temperatures.

NASA—National Aeronautics and Space Administration.

NRC—National Research Council.

Outgassing—The emanation of gases from within an object.

P_g—Probability of growth.

Phenotypic—An organisms observed characteristics or traits that result from the expression of the organism gene, environmental factors, and the interaction between the two.

Photosynthesis—The process by which certain organisms use the energy derived from sunlight to sustain their metabolism.

Pluvial—Processes involving abundant rainfall.

PP—Planetary protection.

Psychrophiles—Organisms that have a maximum growth temperature of 20 °C, an optimal growth temperature of 15 °C or lower, and a minimum growth temperature of 0 °C or lower.

Rad—A measure of radiation exposure defined in terms of the total amount of energy absorbed per unit mass of the absorbing material. One rad is equal to 100 erg of energy deposition per gram of the target material. Because the amount of energy absorbed depends on the nature of the target material, the unit is often qualified to indicate the nature of the target, e.g., 5 krad [water] per month.

Radiation-resistant organisms—Organisms that can survive and grow following acute exposure to radiation.

Radiolysis—The breakdown of molecules as a result of exposure to ionizing radiation.

Redox couples—A coupled series of chemical reactions driven by the simultaneous loss of electrons from one species [oxidation] and the gain of electrons from a second species [reduction].

Regolith—The layer of fragmented, incoherent rocky debris on the surface of a planetary body.

Retrograde—Rotational or orbital motion in the opposite direction to that of Earth.

RNA (ribonucleic acid)—A polymer of nucleotides connected via a sugar-phosphate backbone. It plays an important role in protein synthesis and other chemical activities in cells.

rRNA—Ribosomal RNA.

Spores—A single- celled asexual reproductive unit created by a variety of microorganisms to aid in the dispersal and survival over extended periods of time in adverse environmental conditions.

Sterilization—A procedure that destroys all living microorganisms, including vegetative forms and spores. In practice, a completely sterile state is rarely achieved.

TBD—To be determined.

Tectonism—Processes acting within the lithospheres of planetary bodies responsible for the creation of large scale structures.

Thermophiles—Organisms that can survive and grow in high-temperature environments.

Tidal heating—Heating of a planet or satellite as a result of the work performed on the object's materials by the flexing due to gravitational interactions between bodies.

Trojan asteroids—Asteroids located at the 1/1 mean-motion resonance (commensurability) with Jupiter, librating about the L4 and L5 points 60 degrees ahead of, and behind, Jupiter in its orbit.

TSA (trypticase soy agar)—A solid growth media used to culture microorganisms.

TSSM—Titan Saturn System Mission.

Upwelling—The upward movement of material of a body driven by buoyancy forces as in the case by a low density fluid rising above a high density fluid.

Vegetative bacteria—Bacteria that can grow and reproduce in moist, nutrient rich environments.

VIMS—Visible and Infrared Mapping Spectrometer.