Preventing the Forward Contamination of Europa

Task Group on the Forward Contamination of Europa
Space Studies Board
Commission on Physical Sciences, Mathematics, and Applications
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Foreword

Jupiter's moon Europa is widely regarded as the most promising extraterrestrial habitat for life in the solar system. This view is based on recent evidence suggesting the presence of a water ocean beneath Europa's fractured icy surface together with studies of microbial life in extreme environments on Earth, which suggest that living organisms emerge wherever liquid water and some form of usable energy are found.

Naturally, there is now great interest in learning much more about Europa, primarily through a series of space probes to survey and eventually to land on the surface. But any spacecraft that transports scientific instruments can also carry terrestrial microbes. This report deals with the important issue of how to protect Europa from such inadvertent biological contamination.

To reach their conclusions, the task group had to consider many dimensions of the question. These included techniques for cleaning a spacecraft, the resilience of terrestrial organisms, the space environment at Jupiter, and cost implications of alternative planetary protection approaches. The complexity of these issues, no doubt, contributed to the fact that the task group could not reach consensus on some, but not all, aspects of their charge.

The task group's deliberations illuminate several thorny questions dwelling on the boundary of science and ethics and point the way for further study of both technical and ethical considerations. Although one might have wished for an unequivocal answer, the complexity of the recommendations appropriately reflects the complexity of the underlying issues. In that sense, the report gives NASA's Planetary Protection Officer a very clear message.

Claude R. Canizares, *Chair*
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Preface

Europa, a planetary satellite of Jupiter, is a solar system body that may have a significant potential for past or present life. Europa has a radius of about 1,600 km, slightly less than the Moon's, and it is probably mostly silicate and metal by mass. It has an upper layer, on the order of 80 to 170 km deep, rich in water ice. There is evidence for liquid water beneath the icy crust, first surmised from Voyager data and reinforced by Galileo data. The investigation of Europa's biological potential forms much of the rationale for continued investigation of the satellite.

In accordance with international treaty obligations, the National Aeronautics and Space Administration (NASA) maintains a policy of planetary protection to limit the contamination of extraterrestrial bodies by terrestrial microorganisms and organic compounds during spaceflight missions. Thus, preventing the contamination of Europa's environment by terrestrial organisms will be required during upcoming spaceflight missions such as the orbiter that is currently scheduled for launch later in this decade.

The planetary protection procedures applied to a given spacecraft are currently determined by the nature of its mission (e.g., flyby, orbiter, or lander) and the biological interest posed by the celestial object that is its destination. A lander targeted at an object of great biological interest must undergo careful cleaning, and heat sterilization may be required if it is carrying life-detection experiments. These requirements are, however, specifically tied to the historical development of our understanding of Mars and its biological potential. Given Europa's unique environment, applying the criteria developed for Mars may not be appropriate, and a separate assessment is warranted of the levels of cleanliness and sterilization required to prevent the contamination of Europa by spacecraft missions.

Against this background, NASA's Planetary Protection Officer requested that the Space Studies Board undertake a study to evaluate the planetary protection requirements and methods used to prevent contamination of Europa by terrestrial organisms in future orbiter and lander missions and that it recommend any necessary changes. In particular, the Space Studies Board was asked to do the following:

- Assess the levels of cleanliness and sterilization required to prevent the forward contamination of Europa by future spacecraft missions (orbiters and landers), given Europa's unique environment and our current understanding of terrestrial microorganisms;
- Review methods used to achieve the appropriate level of cleanliness and sterilization for Europa spacecraft and recommend alternatives in light of recent advancements in science and technology; and
- Identify scientific investigations that should be accomplished to reduce the uncertainty in the above assessment.

In response to this request, the Space Studies Board established the Task Group on the Forward Contamination of Europa.

The work of the task group began in early April 1999 and proceeded with a series of meetings and associated presentations, discussions, and deliberations. Despite its best efforts, however, the task group was unable to reach complete agreement on a number of issues. This report describes the majority and minority viewpoints resulting from the task group's deliberations. In particular, it describes their areas of agreement and disagreement and the implications for implementation of planetary protection requirements for future Europa missions.

The work of the task group benefited from contributions, presentations, or comments made by Amy Baker (Technical Administrative Services), Mark Guman (Jet Propulsion Laboratory), Torrence Johnson (Jet Propulsion Laboratory), Harold Klein (SETI Institute), Robert Koukol (Jet Propulsion Laboratory), Jan Ludwinski (Jet Propulsion Laboratory), Christopher McKay (NASA Ames Research Center), William McKinnon (Washington University), Chris Paranicas (Applied Physics Laboratory, Johns Hopkins University), Pericles Stabekis (Lockheed Martin Life Sciences), David Relman (Stanford University), John Rummel (NASA headquarters), Partha Shakkotai (Jet Propulsion Laboratory), Norman Wainwright (Marine Biological Laboratory), and Wayne Zimmerman (Jet Propulsion Laboratory).

This report has been reviewed by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC's) Report Review Committee.

The purpose of this independent review is to provide candid and critical comments that will assist the authors and the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The contents of the review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. The task group thanks reviewers John Battista (Louisiana State University), Russell Doolittle (University of California, San Diego), John Kerridge (University of California, San Diego, retired), Krishan Khurana (University of California, Los Angeles), Leslie Orgel (Salk Institute for Biological Studies), Robert Pappalardo (Brown University), and M. Elisabeth Paté-Cornell (Stanford University) for their many constructive comments and suggestions. Responsibility for the final content of this report rests solely with the authoring task group and the NRC.

Contents

Executive Summary

Planetary protection is an essential consideration for exploration of planets or satellites that may have experienced prebiotic chemical evolution or that may have developed life. Recent observations of Jupiter's satellite Europa indicate that it has been geologically active in the relatively recent past and that liquid water might exist beneath a surface ice shell some 10 to 170 km thick. Moreover, water might exist closer to the surface on an intermittent basis if the ice shell is cracked or otherwise punctured owing to the action of internal and external forces.

We know that life arose rapidly on Earth, perhaps in ancient hydrothermal systems. In these systems, cold ocean water is taken up and circulated through a geothermally heated zone, where it interacts chemically with minerals, and is then released back into the ocean. Its high temperature and dissolved mineral content result in a state of physical and chemical disequilibrium when it mixes again with the cold water. On Earth, the subsequent reactions to reestablish equilibrium were able to provide energy to support metabolism. Europa may also have such geothermal zones if a global ocean of liquid water exists below the surface.

Terrestrial microorganisms provide the only available reference point for evaluating whether life might already be present on Europa or whether it could be introduced by a contaminated spacecraft. On Earth, life is found in some of the most extreme environments. These include extreme heat, cold, pressure, salinity, acidity, dryness, and radiation. Microorganisms are remarkably resilient and have survived exposure to the space environment for more than 5 years aboard the Long Duration Exposure Facility and for millions of years in permafrost regions on Earth's surface. Moreover, in some circumstances, the ability to survive one form of environmental stress may confer the ability to survive in another stressful environment. Many common bacteria are, for example, desiccation resistant, and there is evidence suggesting that the mechanisms that evolved to permit survival in very dry regions also confer resistance to irradiation. Organisms capable of surviving a particular set of extreme conditions cannot, therefore, be assumed to be necessarily confined to environments possessing those conditions.

Even though current information is not sufficient to conclude whether Europa has an ocean, native life, or environments compatible with terrestrial life, it is also insufficient to dismiss these possibilities at this time. Thus, future spacecraft missions to Europa must be subject to procedures designed to prevent its contamination by terrestrial organisms. This is necessary to safeguard the scientific integrity of future studies of Europa's biological potential and to protect against potential harm to europan organisms, if they exist, and is mandated by obligations under the United Nations' *Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, Including the Moon and Other Celestial Bodies* (U.N. Document No. 6347 January 1967).

Current NASA requirements for the protection of other planetary environments are based on categorizing the mission as to type and the target object as to its likelihood of harboring life. The current procedures for planetary protection use protocols derived from those originally developed for the Viking missions to Mars in the 1970s. Determining whether or not this methodology is applicable to Europa missions was the central facet of the task group's deliberations.

The Task Group on the Forward Contamination of Europa concluded that current cleaning and sterilization techniques are satisfactory to meet the needs of future space missions to Europa. These techniques include Viking-derived procedures such as cleaning surfaces with isopropyl alcohol and/or sporicides and sterilization by dry heating, as well as more modern processes such as sterilization by hydrogen peroxide, assuming that final sterilization is accomplished via exposure of the spacecraft to Europa's radiation environment. The technological drawbacks of current prelaunch sterilization techniques are such that the use of such techniques is likely to increase the complexity and, hence, the cost of a mission.

The task group also concluded that the current spore-based culturing techniques used to determine the bioload on a spacecraft should be supplemented by screening tests for specific types of extremophiles, such as radiation-resistant organisms. In addition, modern molecular methods, such as those based on the polymerase chain reaction (PCR), may prove to be quicker and more sensitive for detecting and identifying biological contamination than NASA's existing culturing protocols for planetary protection.

The task group recommends a number of studies that would improve knowledge of Europa and that would better define the issues related to minimization of forward contamination. These include studies on the following topics:

- Ecology of clean room and spacecraft-assembly areas, with emphasis on extremophiles such as radiation-resistant microbes;
- Detailed comparisons of bioload assay methods;
- Desiccation- and radiation-resistant microbes that may contaminate spacecraft during assembly;
- Autotroph detection techniques; and
- Europa's surface environment and its hydrologic and tectonic cycles.

The task group was unable to reach complete agreement on the central issue of the planetary protection standards that must be met by future missions to Europa. The majority if its members believe that Europa's potential importance to studies of chemical evolution and the origin of life is great but that detailed understanding of the europan environment and the survival of terrestrial organisms in extreme conditions is so limited that the current planetary protection methodology is not readily applicable to Europa missions. Uncertainties demand conservatism, and, thus, the very first mission to Europa must meet the highest reasonable level of safeguard.

In practice, this means that the bioload of each Europa-bound spacecraft must be reduced to a sufficiently low level at launch that delivery of a viable organism to a subsurface ocean is precluded at a high level of probability. This approach allows mission planners to take advantage of the bioload reduction likely to occur en route, particularly while in Jupiter's radiation environment. One consequence of this view is that Europa must be protected from contamination for an open-ended period, until it can be demonstrated that no ocean exists or that no organisms are present. Thus, we need to be concerned that over a time scale on the order of 10 million to 100 million years (an approximate age for the surface of Europa), any contaminating material is likely to be carried into the deep ice crust or into the underlying ocean.

Thus, the task group's majority concluded that spacecraft sent to Europa must have their bioload at launch reduced to such a level that, taking into account the natural additional reduction that occurs after launch, the probability of contaminating a europan ocean with a viable terrestrial organism at any time in the future should be less than 10^{-4} per mission. How this standard might be implemented by a combination of Viking-level cleaning and sterilization, accompanied by bioload reduction in the europan radiation environment, is illustrated by a probabilistic calculation offered by the task group (Appendix A).

In addition to the majority view, this report presents two independent minority viewpoints that argue for less stringent planetary protection requirements. *

* The minority viewpoints supporting less-stringent planetary protection procedures than those advocated by the majority are based on two independent arguments. One subset of the task group argued that the planetary protection provisions for Europa should be broadly consistent with the current policies, practices, and protocols. The other subset argued that studies of the organisms found in extreme terrestrial environments suggest that no known terrestrial organism has a significant probability of surviving and multiplying in a europan ocean. The practical consequences of both of these views is that Europa missions should be subject to essentially the same planetary protection requirements that are currently applied to Mars missions. That is, spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level cleaning, but sterilization is not necessary.

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Planetary Protection Policies

HISTORY

Planetary protection concerns were first raised in 1956 in a discussion of the nascent field of space law at the International Astronautical Federation's 7th Congress, held in Rome.[1] Planetary protection became an issue for the scientific community in December 1957 when Joshua Lederberg wrote a letter to the National Academy of Sciences. He was concerned that it would not be possible to test the panspermia hypothesis for the origin of life if the Moon became contaminated with terrestrial organic matter as a result of spacecraft missions.

The Moon was soon found to be barren, however, and interest in panspermia waned.[2] Nevertheless, the importance of preserving extraterrestrial environments from terrestrial biological and organic contamination was generally recognized and ultimately enshrined in resolutions issued by the Committee on Space Research (COSPAR) of the International Council for Science and in the provisions of the United Nations Outer Space Treaty.[3]

As a signatory to the Outer Space Treaty, the United States is obliged to ". . . pursue studies of outer space, including the moon and other celestial bodies . . . so as to avoid their harmful contamination. . . ."[4] To abide by the treaty's imperatives, NASA has developed detailed planetary protection procedures in accordance with general requirements outlined in recommendations in various reports provided by the Space Studies Board. NASA implementation plans are then submitted to COSPAR for that organization's approval in its de facto role as the international court of scientific opinion with respect to the Outer Space Treaty's noncontamination policies.

It is generally acknowledged that minimizing the risk of forward contamination is motivated by two different imperatives.[5] The firt is preservation of the scientific integrity of the planetary body under study. That is, terrestrial organisms introduced into an extraterrestrial environment may cause false positives in life-detection experiments and may generally impede the study of indigenous life, if it exists. The second imperative is to preserve and protect indigenous organisms from possible harm by introduced terrestrial life.

For much of the history of the implementation of planetary protection regulations, the protection of future scientific experiments has been assigned the most weight in determining planetary protection requirements. Indeed, planetary protection policies have centered on the concept of a period of biological exploration, during which particular planetary bodies are accorded protection from contamination so that studies of their biological potential can proceed unhindered by terrestrial contamination. Following the expiration of this exploration period, contamination controls are then relaxed or abandoned altogether.

Over the past 15 years, however, ethical and philosophical papers have been published on the rights of alien beings, no matter how simple those beings. Combined with the emergence of environmental and animal-rights groups, this is a potential area for future debate. Indeed, a 1992 report of the National Research Council's (NRC's) Space Studies Board, *Biological Contamination of Mars: Issues and Recommendations,*[6] recognized these issues and emphasized the need to encourage public discussion and dissemination of information concerning the steps taken to prevent planetary cross-contamination.

PROTECTING MARS

Mars has been the focus of the search for extraterrestrial life for most of the last 40 years. As such, the development and implementation of planetary protection policies have evolved in close concert with the evolution of our understanding of the martian environment and its biological potential. Before the Viking missions of the mid-1970s, the severity of the martian environment was not completely known. The subsequent improvement in understanding is reflected in the fact that the value adopted for the probability of growth of imported terrestrial microbes on Mars (P_g) used in the probabilistic approach to contamination control applicable at that time fell from 1 in 1964 to 10^{-10} in 1978.

The clarification of the biological potential of the martian surface had a major impact on planetary protection policies for Mars and, by extension, other solar system bodies. NASA's current planetary protection requirements and those for Mars, in particular, derive from a policy adopted at COSPAR's 25th General Assembly, held in Graz, Austria, in 1984,[7] as refined in a 1992 NRC report on the topic.[8] The key feature of COSPAR's 1984 policy was the abandonment of the quantitative, statistical approach used in the Viking era and the adoption of a simpler, more straightforward methodology based on the type of mission (e.g., flyby, orbiter, lander, or sample return) and the degree to which the mission's destination is of interest to the process of chemical evolution.

The 1992 NRC report refined the COSPAR approach by drawing a distinction between Mars missions that carry instruments designed to search for evidence of life and those that do not carry them. Since terrestrial organisms are unlikely to grow on the martian surface, the report argued, they do not pose a significant contamination hazard. They could, however, confound the results from life-detection experiments. Thus, the report recommended that landers carrying instrumentation for in situ investigation of extant martian life "should be subject to at least Viking-level sterilization procedures" (see Box 1.1).[9] Orbiters and landers without biological experiments, on the other hand, "should be subject to at least Viking-level presterilization procedures —such as clean-room assembly and cleaning of all components—for bioload reduction, but such spacecraft need not be sterilized."[10] The NRC's recommended distinction between Mars landers with and without life-seeking experiments was later codified and adopted by COSPAR.[11] ,[12]

NASA's implementation of these policies, described in *Planetary Protection Provisions for Robotic Extraterrestrial Missions,* involves adherence to the following procedures: [13]

- *Spacecraft that fly by or enter orbit around Mars* are subject to planetary protection requirements designed to control contamination and to reduce the risk that spacecraft or its boosters will impact the planet. This is achieved by assembling the spacecraft in clean rooms rated at Class 100,000 or better (i.e., less than one particle in the size range 1 mm to 0.001 μm for every 100,000 cubic feet of air) and by ensuring that the probability of impact by the launch vehicle and the flyby spacecraft does not exceed $10^{-4}$and 10^{-2}, respectively. The lifetime of an orbiter must be such that it remains in orbit for a period in excess of 20 years from launch, and the probability of impact for the next 30 years must be no more than 0.05. If the lifetime requirements cannot be met, then the surface microbial bioburden must meet the Viking presterilization limit. Following bioassay, such spacecraft must be protected against recontamination.
- *Spacecraft that land on Mars but are not equipped with life-detection experiments* are subject to planetary protection requirements designed to control the lander's bioburden and to prevent accidental impact by hardware not intended to land. The total probability of any accidental impacts by any hardware other than the lander must be no more than 10^{-4}. Bioburden control involves assembly in a Class 100,000 or better clean room, periodic microbiological assays, and maintenance of hardware cleanliness. Bioburden reduction to the Viking presterilization level is required. The mission team is also required to inventory, document, and archive samples of organic compounds used in the construction of the lander and associated hardware that might accidentally impact the planet. Finally, the locations of landing sites and impact points must be determined as accurately as possible, and the condition of the hardware at each site must be estimated to assist in determining the potential spread of organic compounds.
- *Spacecraft that land on Mars and are equipped with life-detection experiments* are subject to all of the requirements outlined above and must, in addition, undergo a Viking-level sterilization process.

Although the bioburden reduction employed for all types of landers may be measured by any microbiological assay, it is incumbent on the project to prove the equivalency between its assay and that employed on Viking. Moreover, no allowance can be made for burden reduction in flight or associated with surface conditions on Mars.

The central question to be addressed in this report concerns the degree to which Europa can be incorporated into the planetary protection framework developed in light of 40 years of experience with the exploration of Mars. In other words, is our current knowledge of Europa and its ability to sustain terrestrial organisms analogous to our understanding of martian conditions before the Viking missions or after them? In an attempt to answer this question, Chapter 2 focuses on current understanding of Europa and Chapter 3 discusses the limits of terrestrial life.

BOX 1.1 VIKING'S APPROACH TO BIOLOAD REDUCTION

Viking employed a twofold approach to controlling the population of terrestrial organisms that might find their way to Mars. First there was a careful cleaning of the spacecraft, and then the bioload was reduced still further by heat sterilization.

Presterilization

The Viking landers were assembled in Class 100,000 clean rooms. During assembly, thousands of microbial assays were conducted, and these established that the average spore burden per square meter was less than 300 and the total burden of spores on the lander's surface (i.e., the exposed exterior and those parts of the interior communicating directly with the exterior) was less than 300,000.[14] The spore-forming microbe *Bacillus subtilis* was used as the indicator organism in the microbiological assays on the basis of its enhanced resistance to heat, desiccation, and radiation.

Sterilization

Once the landers had been assembled and sealed inside their bioshields, their bioload was further reduced by dry heating. The landers were heated at a humidity of 1.3 mg/liter such that at the coldest point a temperature of 111.7 °C was maintained for some 30 hours. In other words, much of the lander was subject to a higher temperature for a longer period of time. Once sterilized, the landers were no longer accessible for additional microbial assays. Thus the efficacy of the sterilization procedure was estimated indirectly on the basis of the known heat-survival characteristics of *B. subtilis* and was credited with reducing the lander's bioburden by a factor of 10^4.

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2

Europa

Interest in Jupiter's moon Europa has intensified with exciting new findings in the last few years from NASA's Galileo mission, currently in orbit around Jupiter, which has made numerous close passes by Europa. This summary of our understanding of Europa, the proposed strategy for its exploration, and the possibility for indigenous life there follows closely the 1999 NRC report *A Science Strategy for the Exploration of Europa.*[1] The task group quotes and paraphrases liberally from that report in the next sections, and the reader is directed to it for greater detail.

Perhaps the most exciting facet of Europa is that an ocean of liquid water may lie beneath its surface covering of ice. Although there is currently no direct evidence for such an ocean, intriguing indirect evidence has been seen from various spacecraft. Europa's reflectance characteristics indicate that its surface is composed of water ice. Local- and global-scale ice tectonics dominates the geology, with a very large number of cracks crisscrossing its surface. Galileo's gravity measurements show that Europa has a differentiated interior, with the outermost 80- to 170-km layer predominantly water and/or water ice. Europa's magnetic signature suggests the presence of an electrically conducting layer near the surface. The likely explanation is that the water layer is liquid, with dissolved salts providing the ions for electrical conduction. Europa also has an extremely thin atmosphere composed of gases derived from its surface.

High-resolution images of the surface show features that are best or most easily explained as resulting from the presence of at least partially melted material at shallow depths.[2] These features include plates of ice that appear to have rafted to new locations and then frozen in place (Figure 2.1)[3] , [4] and cycloidal cracks that can be interpreted as being due to the effects of diurnal tidal stresses on a surface ice shell decoupled from Europa's interior (Figure 2.2).[5]

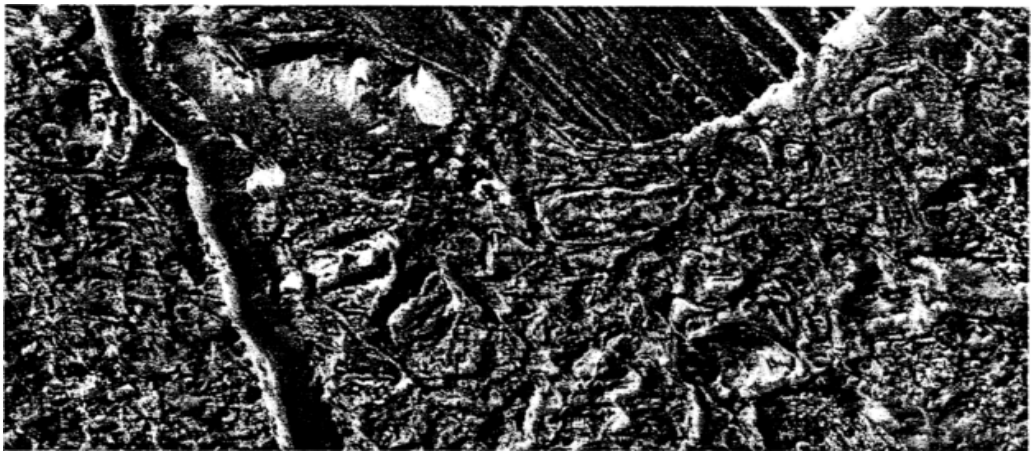

FIGURE 2.1 This view of the Conamara Chaos region of Europa shows an area where the icy surface has been broken into many separate plates that have moved laterally and rotated with respect to each other. North is at the top right of this image, and the Sun illuminates the surface from the east. The area covered by this image, whose center is approximately 8 degrees north and 274 degrees west, is approximately 4 by 7 km. The resolution is 9 m/pixel. Courtesy of the Jet Propulsion Laboratory.

FIGURE 2.2 Europa's cycloidal ridges, such as these seen at 60 degrees north and 80 degrees west, may be the surface expression of diurnal tidal stresses propagating through a thin shell of ice overlying a subsurface ocean. Courtesy of the Jet Propulsion Laboratory.

Because of the likely existence of liquid water, at least as a transient or intermittent species, life could exist within or below Europa's icy shell. The other requirements for life—access to biogenic elements and access to a source of energy—may be available at the water-rock boundary at the bottom of the water layer.[6] Some researchers have argued against this idea on the grounds that Europa's ocean is a closed system and, therefore, its water would rapidly become chemically reduced as the result of interactions with hot rocks in hydrothermal systems,[7] which would mean that a europan ocean would not be an energetically favorable environment for life. This contrary view has, however, been challenged on the grounds that even if the ocean is reducing, abundant redox chemistry could still take place, providing an energy source for metabolism.[8]

Another possibility is that life exists not in the deep oceanic interior of Europa but near the surface. If this is the case, then the ultimate power source for a europan biosphere may be chemical species created by interactions between the surface ice and energetic particles in the jovian radiation environment.[9] While no evidence for life exists, it is the potential for life that makes Europa an exciting target for further exploration.

The most important questions about Europa include whether liquid water exists and whether it has lubricated the motion of surface blocks seen in the Galileo images. Researchers also seek to learn the composition of the deep interior and the non-icy parts of the surface. The nature of the tectonic processes and the abundance of geochemical energy sources are important as well for learning about the potential for—or the history of—life on Europa.

EUROPA EXPLORATION STRATEGY

The important questions about Europa that are outlined above can be addressed through a series of spacecraft missions that carry out the following key measurements, extracted from the 1999 NRC report by the Committee on Planetary and Lunar Exploration (COMPLEX):[10]

Measuring Europa's global topography and gravity, and determining how Europa's shape changes as it orbits Jupiter;
Characterizing Europa's geology and surface composition on a global scale;
Mapping the thickness of Europa's ice shell and determining the interior structure;
Distinguishing between any intrinsic europan magnetic field and induction and/or plasma effects; and
Sampling the geochemical environment of Europa's surface and possible ocean.

COMPLEX concluded that Europa is an exciting object for further study, with the potential for major new discoveries in planetary geology and geophysics, and the potential for studies of extraterrestrial life. In addition, COMPLEX concluded that the results obtained by Galileo (revealing geologically recent or ongoing geologic activity, regarding the possible presence of liquid water, and indicating the potential for present or past biological activity) make Europa a high-priority target for further exploration.

The two highest-priority overall science goals identified in the 1999 report by COMPLEX for Europa exploration reflect the emphasis on the potential for life as a major driver in Europa's exploration. They are the following:

1. Determining whether liquid water has existed in substantial amounts subsequent to the period of planetary formation and differentiation, whether it exists now, and whether any liquid water that is present is globally or locally distributed; and
2. Understanding the chemical evolution that has occurred in the liquid-water environment and the potential for an origin and the possible continued existence of life on Europa.

Even if there turns out to be no life or no sophisticated prebiotic chemistry, these goals remain legitimate drivers for a better understanding of Europa's geologic history.

The particular scientific goals of the first mission are expected to be determination of whether a global ocean of liquid water exists beneath the icy surface, determining, if possible, the spatial and geographical extent of liquid water, determining the bulk composition of the surface material, and characterizing the global geologic history and the nature of any ongoing surface and atmospheric processes. These science objectives can best be met by one or more near-polar-orbiting spacecraft.

EUROPA'S RADIATION ENVIRONMENT

The intense radiation near the surface of Europa is a key factor governing the viability of organisms that might be carried to Europa on spacecraft. Ionizing radiation causes biological effects, such as genetic damage, that result in significant morbidity or death once sufficient damage accumulates. The intensity of the radiation environment in the Jupiter system has been measured by several spacecraft, and its variation with depth below the surface of the ice can be predicted. Accordingly, the rate at which microorganisms with a specified radiation tolerance would succumb in the vicinity of Europa can be determined.

Europa lies deep within the magnetosphere of Jupiter, which is the volume of space above Jupiter's atmosphere that is affected by Jupiter's magnetic field. This magnetosphere extends up to 10 million km from Jupiter (i.e., it encompasses a volume 1,000 times that of the Sun) and is filled with ionizing, magnetically trapped particle radiation. The mechanism of magnetic trapping of radiation at Jupiter is the same as that which operates in Earth's van Allen belts. Jupiter's magnetosphere is, aside from the Sun, the dominant source of energetic charged particles and radio emissions in the solar system.

First discovered as a radio source, the magnetosphere of Jupiter interacts strongly with the innermost Galilean satellite Io, as evidenced by the modulation of decametric radio emissions at the orbital period of Io. The study of Jupiter's decimetric radio emissions led to the first determinations of the approximate strength and direction of the jovian magnetic moment. The first spacecraft visits to Jupiter, in 1973 and 1974 by Pioneer 10 and Pioneer 11, respectively, confirmed the existence of the magnetosphere and revealed its disklike configuration, which rotates approximately with the planet itself. The later visits by Voyager 1 and Voyager 2 in 1979 revealed the importance of Io's plasma torus, a region of sulfur- and oxygen-dominated plasmas maintained by the escape of SO_2 and other S- and O-bearing molecules from Io. The plasma torus mediates the interaction of Io with the jovian

magnetosphere. The Ulysses spacecraft also encountered Jupiter in 1992 and explored the high-latitude, dusk-side magnetosphere whose configuration appears to reflect its interaction with the solar wind. The latest spacecraft to explore Jupiter is Galileo, which began its orbital tour in 1995 and has provided detailed measurements of the interactions of the magnetosphere with Jupiter's satellites as well as synoptic views of global dynamics.

Europa orbits Jupiter at a distance of 671,000 km and is continually bombarded by magnetically trapped, ionizing radiation. This magnetospheric particle flux is the dominant component of the radiation environment at Europa. Galactic cosmic radiation and solar particle radiation cannot access Europa because of Jupiter's magnetic field except at energies exceeding ~90 GeV, where fluxes are negligible.

The radiation flux in the vicinity of Europa varies on many time scales. There are fluctuations that can exceed an order of magnitude with magnetospheric activity over times of minutes. Similarly, smaller fluctuations, typically by a factor of less than two, occur with the 11.2-hour synodic Jupiter rotation period as seen by Europa, because of the variations of trapped particle intensities with magnetic latitude (Jupiter's rotational and magnetic axes are misaligned by 10 degrees). Moreover, variations of up to roughly an order of magnitude have been observed over the 25-year time span between the Pioneer spacecraft encounters with Jupiter and the Galileo mission.

Galileo magnetic field and charged-particle data also imply that the radiation environment varies across the surface of Europa, being only one-fifth as high over the leading hemisphere as over the trailing hemisphere. This happens because the ice surface of Europa absorbs trapped particles as magnetic flux tubes drift across the moon from trailing side to leading side (owing to magnetospheric rotation), depleting the particle population on the leading side.

Data on the radiation environment of Europa have been compiled from information gathered by the Pioneer 10 and 11, Voyager 1 and 2, and Galileo missions. Available measurements include electron intensities in the energy range 30 keV to > 10 MeV and ion intensities from 30 keV to > 100 MeV. Data on ion composition—separation of protons from helium and from ions with atomic number $Z > 6$—are available above about 500 keV/nucleon. These data are input to standard radiation-dose models that calculate the rate of energy deposition versus depth below the target surface.

The results are summarized in Figure 2.3, which shows the radiation dose accumulated at various depths in the europan ice as a function of exposure time. Contributions from jovian electrons, electron bremsstrahlung, and ions are shown. The electron-bremsstrahlung component consists of X rays generated by the high-energy electrons at depth as they are stopped by the target material. The contribution from ions and electrons with energies below 30 keV may safely be ignored even though their energy flux may be significant. This is because particles with such very low energies penetrate Europa's surface to a depth of only about 10 μm for the electrons and even less for the ions.

The units of radiation dose are the rad or the gray (1 rad is 100 erg/g and 1 gray is 1 joule/kg) of energy deposition in the target material. No corrections have been made for the relative biological effectiveness (RBE) of different forms of ionizing radiation, based on the rates of linear energy transfer (LET). In higher organisms, such as humans, it is well established that high-LET radiation (e.g., stopping protons, heavy ions) has a greater biological effect than does low-LET radiation (e.g., electrons and X rays), as reflected by RBE values as high as 20 depending on the specific biological end point. However, no experimentally established RBE values for microbes are available.

Figure 2.3 shows that the radiation environment at 10-cm depth in europan ice is ~5 krad per month. The radiation dose is dominated by electrons and bremsstrahlung over depth values down to approximately 1 meter, below which protons dominate. At greater depths, the radiation environment continues to decrease, reaching values similar to those in Earth's biosphere below depths of 20 to 40 m (not shown). Hence, once microorganisms are transported below a shallow depth at Europa—at most a few tens of meters below the surface—radiation is no longer a significant environmental factor.

For comparison, the natural radiation environment at the surface of Earth gives an average dose of about 0.1 rad per year. The ionizing radiation exposure limits for astronauts are 25 rad per month and 50 rad per year, not to exceed 100 to 400 rad total in a career, depending on age and sex (these are whole-body doses). Many microorganisms tolerate far more radiation; *D. radiodurans,* for instance can grow and reproduce in a 6-krad/hour environment.

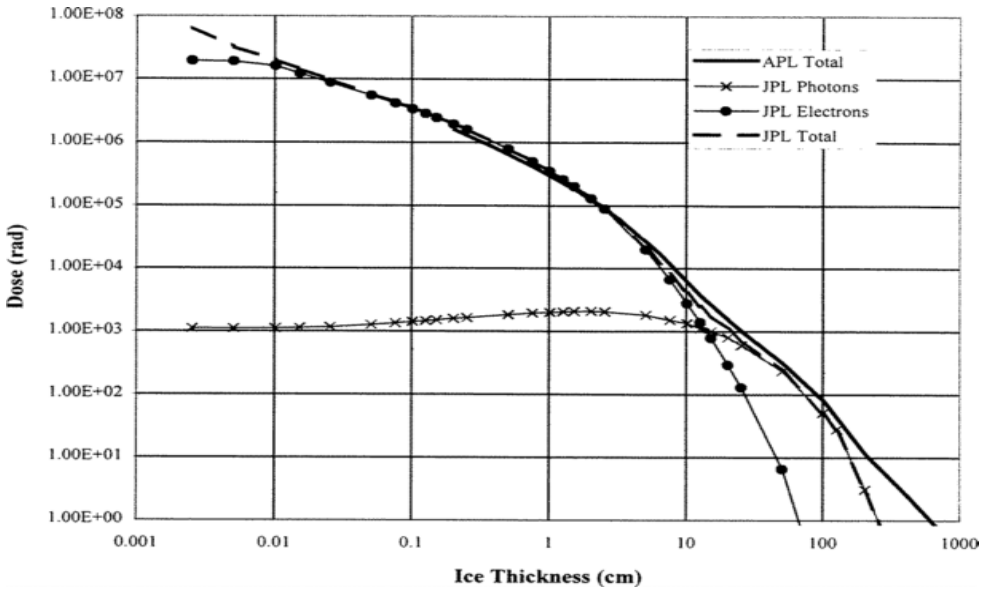

FIGURE 2.3 Radiation dose models for Europa, in rad [water] per month (30.4 days) of exposure below varying thicknesses of ice. The results of two independent evaluations are given, "JPL Total" and "APL Total." For the JPL Total model, the separate contributions of electrons and photons (bremsstrahlung) are shown. The APL Total model has higher proton fluxes at very high energies. In addition to the theoretical uncertainties in Europa's radiation environment (as indicated by the differences between the APL and JPL models), natural variations of up to an order of magnitude have been observed in Jupiter's trapped-particle intensities over the 25-year span between the Pioneer and Galileo missions. Information provided by J.M. Ratliff of the Jet Propulsion Laboratory and C.P. Paranicas of the Applied Physics Laboratory, Johns Hopkins University.

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3

Life in Extreme Environments

A wide variety of extreme environments is known to exist on Earth, including those characterized by, for example, physical conditions such as extreme temperatures and pressures and chemical conditions such as salinity, acidity, or alkalinity. These environments are different from the preferred environment to which we human beings are adapted, yet they are inhabited by organisms that have successfully adapted to them. This chapter discusses some of these organisms, collectively known as extremophiles, and some of the environments they inhabit. Such information can provide insights into the types of terrestrial organisms that might survive and grow on Europa.

EXTREMOPHILES

Extremophiles can be categorized according to the physical characteristics of the environments in which they live. Thus, for example, the thermophiles and barophiles are found in regions characterized by high temperatures and pressures, respectively.1 The organisms most relevant to an assessment of the probability of the contamination of Europa are those capable of surviving in environments characterized by some combination of low temperatures, high pressures, and a high background radiation.

Psychrophiles and psychrotrophs are types of microorganisms that inhabit cold environments. Psychrophiles have a maximum growth temperature of 20 °C, an optimum growth temperature of 15 °C or lower, and a minimum growth temperature of 0 °C or lower,2 whereas psychrotrophs have somewhat warmer optimum and maximum growth temperatures. While both are found in many cold environments, only psychrotrophs are found where the temperature can exceed the maximum growth temperature of the psychrophiles in question. In many polar environments, for example, radiant energy can increase the temperature above the maximum growth temperature for psychrophiles, and as a result they expire. This is probably the main reason no psychrophiles are found in the terrestrial portions of Antarctica. Their abnormal susceptibility to warm temperatures means that they are unlikely to be present in a spacecraft-assembly environment and are therefore unlikely to contaminate a spacecraft.

Psychrotrophs, on the other hand, can be found in any environment, and most of the early research on these organisms was carried on by food microbiologists. Various species of psychrotrophs are in well-known genera such as *Pseudomonas, Flavobacterium, Achromobacter, Alcaligenes, Bacillus, Arthrobacter,* and *Vibrio.* Microbial ecologists have found them in most cold environments, even in the harsh desert environments of Antarctica.3 These organisms can also be found in permafrost as well as in the deeper layers of ice cores, indicating that they have the ability to survive for extremely long periods of time. Laboratory studies indicate that not all species can survive the freezing and thawing process, and many species are killed when frozen, especially if they are in the exponential growth phase.

The best estimate for the minimum temperature of microbial growth is -10 to -12 °C (although there are a few reports of growth at lower temperatures), and this low temperature has been recorded for only a few bacteria. This minimum temperature for growth appears to be determined by the fluidity of cell membranes and the availability of liquid water. If an organism cannot desaturate its membrane lipids, the cellular transport of substrates ceases. The freezing property of the liquid within and immediately adjacent to the cell also comes into play. Either factor can prevent the cell from growing. It is extremely doubtful that any organism can grow at 100 K, but survival remains a possibility at any depth in Europa's ice.

Psychrotrophs may survive at the surface temperatures of Europa, as indicated by current techniques that employ freezing for preserving microbial cells. Europa's geologic conditions may not change significantly for a long period of time and so the microbes might have to stay in this survival state for millions of years. Surviving microbes might have extreme difficulty initiating growth owing to the absence of organic matter for heterotrophic growth and their inability to metabolize at 100 K. The absence of an organic matter energy source does not, however, rule out the possibility of psychrotrophic chemoautotrophic growth if the organism can reach subsurface liquid water.

Barophiles are microorganisms that thrive under conditions of high hydrostatic pressure, and all known examples inhabit marine environments. Europa's ice shell may be 10 to 170 km thick, and thus the pressure in the upper layers of a hypothetical europan ocean would have to be 13 to 210 MPa (130 to 2,100 bars), assuming that the ice has the same density as water. Studies indicate that most organisms cannot grow when the pressure exceeds 60 MPa, and many are indeed killed at that pressure. Known terrestrial organisms could withstand the pressure near the top of Europa's putative ocean, especially if the ice shell is relatively thin. A combination of high pressure and low temperature would, however, decrease the molecular volume of a microbe's macromolecules and would probably bring about its death. For example, *Bacillus subtilis,* one of the indicator organisms currently used to determine the bioload on a spacecraft (see Chapter 5), can survive pressures of 30 MPa provided that the temperature is greater than 20 °C.

Radiation-resistant organisms are of particular relevance to any discussion of the forward contamination of Europa. The bacterium *Deinococcus radiodurans,* for example, can grow continuously, without mutation or any effect on its growth rate, in the presence of 6,000 rad/hr (a dose rate found 1 mm beneath Europa's surface ice). [4] This organism can also survive acute exposures to ionizing radiation of 3 Mrad (at -70 °C) without lethality—a dose that induces about 130 double-strand breaks (DSBs) per chromosome. Furthermore, viable cells are readily recovered from cultures even after exposure to 8 Mrad (at -70 °C).[5] This ability is extraordinary since most cells cannot survive irradiation at more than 500 to 100,000 rad[6] or 1 to 3 DSBs per haploid chromosome.[7] Recent advances have led to insights into this bacterium's exceedingly efficient DNA repair capabilities,[8,9] which have been shown to be partly responsible for its resistance to radiation.[10,11]

Because there are no known radioactive environments that can explain the evolution of *D. radiodurans*'s resistance to radiation, there is general agreement that this organism's resistance to radiation is a secondary characteristic developed in response to some other environmental stress. The consensus view is that the mechanisms that evolved to permit survival in very dry environments also confer resistance to radiation.[12]

It is possible that other desiccation-resistant microorganisms, not yet described as radiation-resistant, could pose a threat to the europan biosphere. Such organisms can only pose a threat if they can survive a multi-year journey to Europa. Despite the discrediting of the oft-repeated claim that live bacteria were recovered from Surveyor 3's camera after surviving on the Moon's surface from April 20, 1967, to November 20, 1969, experiments conducted aboard a variety of spacecraft including the European Retrievable Carrier and the Long Duration Exposure Facility indicate that a variety of common terrestrial bacteria are able to withstand the space environment for periods as long as 6 years.[13,14] Since the radiation-resistance characteristics of many common organisms (and most extremophiles) are unknown, it is conceivable that many bacteria classified as desiccation- and/or radiation-resistant will survive in extraterrestrial environments.

On Europa, life-sustaining, near-surface environments may exist within or under regions of water ice, since ice will provide microbes with some degree of radiation protection. In addition to requiring water in the liquid state, genetic repair would certainly also be dependent on the presence of a source of carbon and energy. There is currently little or no evidence for any organic matter on Europa's surface due to the lack of observable spectral features of CH bonds in Galileo's infrared spectra of Europa. Nevertheless, the presence of carbon in material recycled from the interior via geologic processes or in cometary and meteoritic debris cannot be discounted.[15]

EXTREME ENVIRONMENTS

The ability (or inability) of terrestrial organisms to adapt to, and survive and multiply in, extreme terrestrial environments reveals much about the resilience of life in stressful circumstances. Given that these organisms have had millions of years to come to terms with their particular physical and chemical environments, their ability to cope provides some insight into the problems facing terrestrial organisms suddenly introduced into extraterrestrial environments. Earth's polar regions present two particularly telling examples, the cryptoendolithic environments found in the polar deserts and permafrost.

Antarctic cryptoendolithic environments exist where communities of microorganisms have colonized the surface layers of porous rocks to depths of a few millimeters. Photosynthetic members of the community utilize sunlight that penetrates the translucent rock crust. The ambient air in the Antarctic desert is rarely above 0 °C, but the near-surface regions of rocks exposed to the Sun are warmed by solar radiation. The cryptoendolithic colonies obtain water from snow, which melts when it falls on warm and dry rock surfaces.

The cryptoendolithic environments are good examples of absolute extreme environments, i.e., regions where the physical conditions are beyond adaptability. The organisms colonizing the rocks are not adapted to their environment; they survive by tolerating it. While all metabolic activity occurs at ~±10 °C, the optimum temperatures for organisms, as measured in the laboratory, range from 15 to 25 °C, temperatures rarely reached in the Antarctic. Thus microorganisms in Antarctic rocks live near the lower limits of their physiological potential, and they have no reserves to compensate for changes in the environment, should conditions deteriorate. As a consequence, even a minor change in climate can result in local extinctions. In fact, close to 80 percent of the cryptoendolithic communities in Antarctica are dead or fossilized.[16]

Permafrost microorganisms have been studied most extensively in Siberia[17] and have recently been found in Antarctica.[18] Permafrost microorganisms originate in the soil where they have been immobilized by freezing, while new soil continues to be formed on the surface. In Siberia, the oldest permafrost is 3.5 million to 5 million years old. Recent drilling in Antarctica revealed permafrost some 8 million years old. Permafrost temperatures are extremely stable, around -10 °C in Siberia and down to about -30 °C in Antarctica.

The number of viable bacteria (up to 10 million colony-forming units per gram of dry weight) and the abundance of species found in permafrost decrease with increasing depth (i.e., with increasing age). The viable microbial community in permafrost—mostly psychrotrophs and only very few psychrophiles—is dominated by prokaryotes (organisms whose cells lack a nucleus). Eukaryotic algae (i.e., algae whose cells contain a nucleus) do not survive beyond 5,000 to 7,000 years, but viable yeasts are found in 3 million-year-old permafrost. The composition of bacterial communities found in permafrost mirrors that of the soil from which they originate. Most bacteria isolated from permafrost are aerobes; only a few are anaerobes, mostly methanogens. Permafrost at -10 °C and below is frozen solid. Yet a thin film of unfrozen water envelopes both the inorganic soil particles and microorganisms. The thickness of this unfrozen water film is temperature-dependent and is reduced to about 0.5 nm at -5 °C and below.

In permafrost, microbial growth is in a stationary phase and cell division probably does not occur. This, together with the fact that the number of species decreases with age, suggests that in permafrost a slow selection process takes place, and bacteria that are not able to tolerate the physical conditions of their environment eventually become extinct. In permafrost there is no adaptation, only selection.

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4

Sterilization and Cleaning Methods

The procedures used to ensure spacecraft cleanliness and, ultimately, to achieve the desired sterilization standards begin during the design and manufacturing of spacecraft components. Afterward, when the components are being assembled, further cleaning and sterilization protocols are implemented. Unfortunately, it is not currently practical to sterilize an entire spacecraft at one time, post-assembly, while at the same time protecting all of its diverse components and sensors from damage or failure. The different sensitivities of internal components to sterilizing procedures require that many of the parts be sterilized individually, using a procedure compatible with their function. For complex scientific missions, therefore, whole-spacecraft sterilization is not an option—a single sterilization procedure would be limited by the spacecraft's most sensitive component. As a result of this constraint, many spacecraft components are sterilized individually and then assembled in clean rooms using rigorous procedures that minimize recontamination.

CLEANING AND STERILIZATION STANDARDS

NASA's current planetary protection requirements for Mars missions are derived from the procedures applied to the Viking landers. Missions not carrying life-detection experiments must be cleaned to ensure that the spacecraft's total bioload does not exceed 300,000 spores and that the density of spores on the spacecraft's surfaces does not exceed 300 m**-2.**Missions with life-detection experiments must undergo additional procedures to ensure that the total bioload does not exceed 30 spores. The effectiveness of the various procedures currently used by NASA and its contractors to meet these bioload standards is determined by the use of reference organisms, including **Bacillus subtilis** (var. *niger*), *Bacillus pumilis,* and *Bacillus stearothermophilus. Bacillus spp.* (endospore formers) were originally selected as a microbiological indicator of sterilization success on the basis of their enhanced resistance to heat, desiccation, and radiation.

ACHIEVING THE STANDARDS

The twofold approach to the control of forward contamination used by the Viking mission—careful cleaning of the spacecraft, followed by active bioload reduction through heat sterilization (see Box 1.1 in Chapter 1)—forms the basis for the procedures currently in use. All missions are carefully cleaned and then those with life-detection experiments undergo sterilization.

The Viking landers were assembled in clean rooms (see Box 4.1 for a description of current clean-room procedures). During assembly, microbial assays (see Chapter 5) were conducted to establish that the average and total burden of spores on the lander's accessible surfaces were 300 m $^{-2}$and 300,000, respectively.[1] Current practice requires that those parts of the spacecraft not meeting the requisite bioload standards be washed with isopropyl alcohol and/or a sporicide (ethanol, 65 percent; isopropanol, 30 percent; and formaldehyde, 5 percent) to reduce their bioburden. Decontaminated surfaces are then retested for their contaminating microbiological burden.

Once the landers had been assembled and sealed inside their bioshields, the bioload was further reduced by dry heating the whole spacecraft to at least 111.7 °C for some 30 hours. This procedure was credited with reducing the lander's bioburden by a factor of 10^4. Future spacecraft can be designed to maximize accessibility of their components for pre- and post-assembly bioload reduction. However, some components are hermetically sealed before assembly, so cleaning/sterilizing procedures must be conducted before sealing to prevent recontamination. The sterilization procedures commonly applied in a variety of applications to sealed and unsealed components are listed in Table 4.1. It is worth noting that many of these procedures can have negative impacts on spacecraft performance and may increase mission cost.

TABLE 4.1 Common Sterilization Procedures

Procedure—Target	Techinique—Problems
Dry heat—exterior/interior	105-180 °C for 1 to 300 hours—Problems caused by thermomechanical incompatibility between materials can lead to the faliure of electronic components.
Wet heat—exterior/interior	120-134 °C for 3 to 20 minutes—Problems can be caused by steam (e.g., corrosion and water absorption).
Alcohol wipes—exterior surfaces	Isopropyl or ethyl alcohol swabbing—Problems arise because interior and encased surfaces (e.g., electronic components) are inaccessible.
Ethylene dioxide—exterior/internal exposed surfaces	Toxic gas, 40 to 70 °C—Problems arise because the gas can only reach exposed surfaces and because it is absorbed by some types of polymers (e.g., rubbers and polyvinyl chloride).
Gamma radiation—exterior/subsurface	Typically, 2.5 Mrad—Problems encountered include optical changes in glasses and damage to electronics and solar cells.
Beta radiation—exterior/near-surface	1 to 10 MeV—Problems arise because of limited penetration.
Hydrogen peroxide plasma—exterior/internal exposed surfaces	6 mg/l H202 concentrated at 58%—Problems can be encountered because the unexposed surfaces remain untreated.
Ultraviolet—exterior surfaces	5,000 to 20,000 J/m^2—Problems arise because unexposed surfaces remain untreated.
Methyl bromide—exterior/internal exposed surfaces	Toxic gas—Problems can be encountered because unexposed surfaces remain untreated and because the gas catalyzes chemical reactions between metal and other components.

These treatments can be highly effective, but they have their limitations in certain circumstances. Important factors influencing germicidal activity include the following: the types of microorganisms; the number of organisms; the intrinsic resistance of the organisms; the amount of organic soil on the item to be sterilized; the type and concentration of germicide; the time and temperature of exposure; and the compatibility between of the device being sterilized and the technique being used.

BOX 4.1 CURRENT CLEAN-ROOM PROCEDURES

Clean rooms are highly controlled environments accessible only to trained personnel following strict and unambiguous cleanliness protocols. Representative standard NASA clean-room protocols include the following:

- During assembly, workers are required to wear full face shield suits;
- No human contact directly with spacecraft is permitted. Latex gloves are worn in the clean room, and spacecraft are *not* seeded with tracer organisms to facilitate monitoring;
- Cameras are used to observe and monitor assembly;
- Clean-room air passes through high efficiency particulate air (HEPA) filters and dehumidifiers to minimize airborne microbial contamination and corrosion, respectively;
- Surface particles are removed by vacuuming;
- Witness plates are regularly collected and stored;
- Contact between hardware and biologically relevant materials is minimized; and
- Surface areas of the spacecraft are monitored periodically for their microbiological burden, during and after assembly. Sterile cotton swabs are used to collect contaminating surface microorganisms, which are subsequently cultured and counted.

Unfortunately, clean rooms do not guarantee contamination-free assemblies. Mistakes happen, and clean hardware may not remain clean. Thus, good in-process cleaning procedures are necessary.

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5

Microbial Detection and Identification

A key aspect of planetary protection is the determination of the bioload on the spacecraft prior to launch. From the Viking era to the present day, the bioload has been monitored by a routine and ongoing procedure that continues until shortly before launch. The monitoring entails swabbing accessible external and internal surfaces of the spacecraft with cotton and then determining the number of culturable bacteria from a known surface area. The standard procedure is to transfer the cells from a swabbed surface to a liquid medium, which is then heat shocked at 80 °C for 8 minutes. The surviving cells are then cultured to determine the number of colony-forming units (cfu). A cfu is defined as a colony on a culture plate (using a standard growth medium) that develops from a cell that survived heat shock.

Although the techniques employed in NASA's planetary protection protocols have remained virtually unchanged for the last 25 years, the methods now available for bioload characterization have changed dramatically thanks to advances in biotechnology. It is now possible to survey with good confidence the microbial diversity on a spacecraft or within its assembly area using explicit molecular criteria. A molecular approach circumvents many of the problems associated with culture-based characterization (e.g., delays caused by the time needed to grow the microbial colonies or by the inability to successfully culture most microbes).

The most established methods are based on selective recovery and sequencing of genes encoding the ribosomal ribonucleic acids (RNA).[1] In addition to new technologies such as high-density deoxyribonucleic acid (DNA) hybridization arrays,[2] refinements of existing molecular methods (e.g., the polymerase chain reaction (PCR)) and analytical methods (e.g., mass spectrometry and immunochemistry) provide improved detection capability using a variety of diagnostic cellular biopolymers, including proteins, lipids, and carbohydrates. A variety of advanced detection methods are now in common use. [3] ,[4] Improvements in the sensitivity and specificity of microbial detection should be incorporated into new assessment standards.

It is important to bear in mind that most DNA-based techniques do not necessarily distinguish between living and dead materials. The preservation of DNA in some noncellular contexts is well known, and DNA bound to surfaces such as clay is resistant to degradation.[5] Nevertheless, other biopolymers, such as RNA and phospholipids, are much less stable and generally degrade rapidly following cell death. [6] For example, reverse transcriptase PCR (RT-PCR) has been used to confirm that microbial populations detected at the DNA level are metabolically active.[7] Also, viable microbes have an intact membrane that contains phospholipids. Cellular enzymes hydrolyze the phosphate group from phospholipids within minutes to hours of cell death.[8] Therefore, determination of the total amount of phospholipid ester-linked fatty acids (PLFA) in a particular sample provides a quantitative measure of the viable or potentially viable biomass.[9] Research is needed to develop other techniques able to discriminate between living and dead material.

Acceptable bioburden standards for spacecraft should consider both total abundance and the presence of specific physiological groups. Standards should be defined that take into account the likelihood of survival during transit from Earth and dispersal following landing or impact. Of concern is the possible transport of viable cells to environments supportive of growth of particular classes of terrestrial organisms.

Among the terrestrial microorganisms of most concern are those deriving all of their carbon and energy requirements from inorganic compounds (the chemolithoautotrophs), since they would be most likely to proliferate in a europan ocean. Given current understanding of Europa, it is not unreasonable to suggest that reduced chemical species (e.g., H_2, HS-, and Fe^{+2}) might be produced by geothermal processes within an ocean and that oxidized species (e.g., O_2, CO_2, SO_3-, and SO_4-) might be transported by geologic activity from the surface to an ocean. A variety of recognized chemoautotrophs are capable of growth using these chemical species as substrates for energy generation and growth.

Represented by Archaea and Bacteria, chemoautotrophs are phylogenetically diverse. Although some lineages are composed solely of chemoautotrophic representatives (e.g., methanogens), others (such as the homoacetogens) are intertwined with heterotrophic lineages and could not be easily recognized by phylogenetic affiliation alone. The identification of these lineages may therefore need to consider the presence of key enzymes or genes required for chemoautotrophic growth. For example, genes encoding enzymes for CO_2 fixation (e.g., ribulose bisphosphate carboxylase or carbon monoxide dehydrogenase) are possible diagnostic targets.

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6

Recommended Planetary Protection Strategy for Europa

The task group considered and discussed the history of planetary protection, the particular characteristics of Europa, and the variety of life on Earth in order to develop a set of requirements for the planetary protection of Europa. Unfortunately, the task group was unable to reach a complete consensus on these requirements. Although a majority of the members did agree on a specific requirement, a rationale, and a proposed procedure for meeting that requirement, two significant independent minority views were also expressed.

This chapter outlines the task group's recommended planetary protection strategy for Europa and goes on to explain the areas of general agreement as well as the points on which the views of the two minority subsets diverged from that of the majority. The consequences of these viewpoints are described along with the arguments pro and con.

The task group is in complete agreement that planetary protection is an important goal for all space missions. Limiting the forward contamination of Europa is necessary to preserve the scientific integrity of future biological studies and to protect any indigenous life forms. NASA has a scientific, moral, and legal responsibility to take this task seriously, even if living up to these responsibilities is costly. The task group is also in complete agreement that the current evidence for the existence of a global ocean is persuasive but not definitive. And, of course, even if there were definitive evidence for an ocean, it would still be premature to assume the presence of indigenous biota on Europa.

PLANETARY PROTECTION STRATEGY FOR EUROPA

Given the uncertainties in our knowledge of the diversity of life on Earth and the recent discoveries of organisms living in extreme environments, the majority of the task group believes that a conservative approach must be taken to protecting the europan environment. Furthermore, since Europa, unlike Mars, may have a global ocean, a viable organism could colonize the entire subsurface via the ocean connection.

Although it is premature to conclude that either an ocean or biota exist on Europa, it is prudent to implement planetary protection procedures that assume the existence of both. In this case, we are obliged to protect the europan environment for an open-ended period, until it can be demonstrated that no oceans or organisms are present. This viewpoint mandates that the bioload on *any* spacecraft sent to Europa must be reduced to such a level that the probability of inadvertent contamination of a europan ocean by viable organisms is very low, either in the next 100 years or at any time in the future.

The task group therefore recommends the following standard: for every mission to Europa, the probability of contaminating a europan ocean with a viable terrestrial organism at any time in the future should be less than 10^{-4} per mission. This standard calls for explicit calculation of the probability of contamination posed by each particular mission. It allows spacecraft designers to take advantage of the bioload reduction that occurs from radiation in the jovian environment (see Chapter 2). The value of 10^{-4} was chosen because of its historical precedents in the planetary protection resolutions issued by COSPAR.[1]

NASA must devise a method for carrying out this calculation. An example of how such a calculation might be done is given in Appendix A. The task group's suggested methodology subdivides the bioload into common microorganisms, spores, radiation-resistant spores, and highly radiation-resistant nonspore microorganisms (e.g., *Deinococcus radiodurans*; see Chapter 3). Assays of the spacecraft and its assembly environment would determine the abundance of these organisms. Multiplying the various survival factors by the probability that an organism will reach the global ocean and grow provides an overall probability that must be less than 10^{-4} in order for a mission to meet this standard.

The task group emphasizes that the sample calculation is intended purely to illustrate a methodology NASA could use to certify that a particular mission meets the 10^{-4} standard. The task group lacks the time, the resources, and the expertise to establish definitive values for each and every parameter considered in the calculation. Instead, the task group has chosen plausible but conservative values for each parameter considered in the calculation. Similarly, it makes no attempt to include either the uncertainties associated with the parameters

entering into the calculation or the possibility that some parameters may be correlated. Indeed, the task group explicitly assumes that all factors are independent.

NASA may decide that more detailed calculations and considerations are necessary or that the calculations for a particular mission show that the probability threshold of 10^{-4} is exceeded at some high level of confidence, given the error bars estimated for the various factors. Future studies such as those recommended in Chapter 7 will naturally change the numerical values in the required calculation.

MAJORITY AND MINORITY VIEWPOINTS

The task group was unable to reach complete consensus on a number of issues relevant to determining the appropriate planetary protection requirements for Europa. Two independent minority viewpoints were expressed by two subsets of the task group. Recognizing that reasonable people may disagree on the interpretation of complex scientific issues, the task group presents here the majority viewpoint and both minority viewpoints so that they may be discussed and retained for the historical record.

Applicability of the Current Planetary Protection Strategies to Europa

The first point of disagreement concerned the applicability to Europa of the current approach to planetary protection as recommended in NRC reports, as adopted by NASA, and as ratified by the international scientific community, embodied by COSPAR. According to this approach, the planetary protection measures applicable to a particular spacecraft depend on the type of mission envisioned and the degree to which its destination is of interest to studies of the processes of chemical evolution and/or the origin of life (see Chapter 1). Application of this methodology requires some detailed knowledge about the object to which the spacecraft is being sent.

One minority subgroup expressed the view that the current strategy of protection via categorization is broad enough to be applicable to Europa. Indeed, this approach has already been applied to recommendations for the prevention of back contamination when europan sample are returned to Earth.2 The implication of this minority view is that the first missions to Europa should be subject to a somewhat augmented version of the protocols currently applied to Mars missions. Thus, orbiters and simple landers would be subject to Viking-level cleaning, while landers with life-detection experiments and/or deep penetrators would be subject to a stricter Viking-level sterilization procedure. Suggested augmentations to the existing cleaning protocols for Mars missions would include assaying for radiation-resistant microbes in addition to spores and the use of molecular-based, cell-detection methods in addition to conventional culturing techniques.

The majority viewpoint is more conservative and argues that Europa must be treated as a special case. The basis for this viewpoint is the current relative ignorance of Europa's possible biology, its possible subsurface ocean—which could allow life to be globally connected—and its possible geologic activity, which may recycle surface material into the ocean on a time scale comparable to the age of the surface, and may also provide a source of chemical energy in the form of organic debris and inorganic substrates entrained from the surface. The majority viewpoint is also based on the possibility that an impacting spacecraft could implant debris sufficiently deep within the ice that it would be protected from radiation.

Survival of Terrestrial Microbes on Europa

The second point of disagreement within the task group concerned the likelihood of the survival and proliferation of a terrestrial organism that might reach a europan ocean (see F_7in the sample calculation contained in Appendix A).

This minority subset of the task group argued that while it is just conceivable that a terrestrial organism might survive in an oceanic environment on Europa, experience from studies of extreme terrestrial environments suggests that such an organism's ability to grow *and* multiply—the real danger to future scientific studies and, potentially, to the survival of indigenous organisms, if they exist—is indistinguishable from zero.

This second subset asserted that no known terrestrial organisms could survive the successive assaults of cold, aridity, and radiation likely to be experienced in transit from Earth to Europa and then finally proliferate in a saline, oceanic environment under extreme hydrostatic pressure. They believe that the combination of physical and

chemical extremes on Europa has no counterpart on Earth, so that no terrestrial organism could have adapted simultaneously to all of them.

Although less than 1 percent of all living species have been characterized to date, both the physiological ecology and the behavior of microbial communities, as well as the environments to which terrestrial microorganisms can adapt, are reasonably well studied. The minority maintained that the known facts are sufficient to form scientifically valid conclusions about the survival and proliferation of terrestrial organisms on Europa.

It argued further that even if organisms that had simultaneously adapted to all the extreme environmental parameters on Europa did exist on Earth, the probability that a spacecraft would be contaminated with significant numbers of these organisms is infinitesimally small. This minority subset would nonetheless be willing to subject future Europa missions of all types to the Viking-level cleaning procedures, so as to significantly reduce their initial bioload.

A majority of the members of the task group did not accept these views. They recommended a more conservative approach and set the probability of proliferation at the relatively small, but finite, value of 10^{-6}(see F_7 in the Appendix A). They argued that prudence is necessary given the variety of life seen in extreme environments on Earth, our ignorance of the extremes of life's adaptability, and our lack of knowledge of the europan ocean. As we learn more, F_7, like the other probability factors discussed in Appendix A, may be updated.

CONCLUSIONS

The majority viewpoint is that a common standard should be set according to which, for every mission to Europa, the probability of contaminating a europan ocean with a viable terrestrial organism at any time in the future should be less than 10^{-4}per mission. NASA would establish the assays and calculations for confirming this figure. The two independent minority viewpoints would both allow future missions to Europa to be governed by the (possibly updated) standards for planetary protection of Mars.

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7

Conclusions and Recommendations

As a result of its deliberations, the task group was able to reach the following conclusions on appropriate planetary protection measures for future missions to Europa. All statements express consensus views unless noted otherwise.

1. To meet planetary protection requirements and obligations, each Europa-bound spacecraft must be cleaned, sterilized, and/or subjected to sufficient radiation prior to contact with Europa's surface so that the probability of contaminating a possible europan ocean with a viable terrestrial organism at any time in the future is less than 10^{-4}.*
2. Current cleaning and sterilization techniques are satisfactory to meet the needs of future space missions to Europa. These techniques include Viking-derived procedures such as washing surfaces with isopropyl alcohol and/or sporicides and sterilization by dry heating, as well as more modern processes such as sterilization by hydrogen peroxide, assuming that final sterilization is accomplished via exposure of the spacecraft to Europa's radiation environment. The technological drawbacks of current prelaunch sterilization techniques are such that the use of these techniques is likely to increase the complexity and, hence, the cost of a mission.
3. The current culture-based method used to determine the bioload on a spacecraft should be supplemented by screening tests for specific types of extremophiles, such as radiation-resistant organisms.
4. Modern molecular methods, such as those based on the polymerase chain reaction (PCR), may prove to be quicker and more sensitive for detecting and identifying biological contamination than NASA's existing culturing protocols for planetary protection.

Knowledge of the planetary protection requirements for Europa will be enhanced by the data returned from future missions and the continuing analysis of Galileo observations. The task group recommends that, in addition, a series of scientific and technical investigations be conducted to reduce uncertainty in calculating the probability of contaminating Europa as a result of spacecraft missions. These investigations include targeted research in the following areas:

- *Ecology of clean room and spacecraft-assembly areas, with emphasis on extremophiles such as radiation-resistant microbes*—Research on the variety and abundance of such organisms in these areas will allow targeting bioload-reduction techniques to the specific organisms present in these artificial environments.
- *Detailed comparisons of bioload assay methods*—What are the strengths and weaknesses of the various types of molecular-based techniques? Quantitative results should be compared in order to determine which methods can best extend current culturing techniques. Can quick PCR methods replace culture-based assays? Can improved detection techniques be developed to readily distinguish between living and dead organisms?

* Two minority views were expressed by two subsets of the task group. Both would allow future missions to meet (possibly updated versions of) the planetary protection standards currently applied to Mars missions. These minority viewpoints are based on two different arguments. One minority subset argued that the planetary protection provisions for Europa should be broadly consistent with the current practice of categorization based on the type of mission (e.g., flyby, orbiter, or lander) and the degree to which the spacecraft's target is of interest to studies of processes related to chemical evolution. The other minority subgroup argued that studies of the organisms found in extreme terrestrial environments suggest that no known terrestrial organism has a significant probability of surviving and multiplying in a europan ocean. The practical consequence of both of these views is that Europa missions should be subject to essentially the same planetary protection requirements that are currently applied to Mars missions. That is, spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level cleaning, but sterilization is not necessary.

- *Desiccation- and radiation-resistant microbes*—These are the microbes most likely to survive the trip to Europa. What is their abundance, their survivability, and their capacity for growth in possible europan environments?
- *Autotroph detection techniques* —Chemoautotrophs are the terrestrial organisms most likely to colonize a deep ocean on Europa. Since these organisms are not easily cultured, which methods give the most sensitive and reliable estimates of their abundance?
- *Europa's surface environment, and its hydrologic and tectonic cycles*—What are the chemical, thermal, and radiation characteristics of Europa's surface? What are the transport mechanisms and their time scales? Does recycling occur, and on what spatial and temporal scales? Does Europa possess a groundwater or hydrothermal system? These studies may provide information on the locations where contamination is unlikely and the prime locations in which to search for indigenous life.

Appendixes

A

Calculating the Probability of Contamination, P_C,

In the main text of this report the task group concluded that for each mission to Europa the probability of contaminating a europan ocean with a viable terrestrial organism at any time in the future should be less than 10^{-4} per mission. The task group offers the following calculation as an example of a methodology NASA can use to certify that a particular mission meets the 10^{-4}standard. The calculation is based on several important assumptions and caveats, including the following:

- The values assigned to individual parameters are not definitive;
- Parameter values are plausible, but err on the side of conservatism;
- No attempt was made to account for uncertainties in the parameters;
- All parameters are assumed to be independent and uncorrelated; and
- The values of particular parameters will change as new information is gathered.

NASA may decide that more detailed calculations and considerations are necessary or that the calculations for a particular mission show that the probability threshold of $10^{-4}$is exceeded at some high level of confidence. Whichever the case may be, the onus is on NASA to determine values of the various parameters in the calculation and to certify them as part of the planetary protection plan for each Europa mission.

Expressing the 10^{-4} standard in terms of the delivery of viable organisms to an ocean allows spacecraft designers and mission planners to take advantage of the bioload reduction attributable to the high radiation environment that occurs naturally in transit to Jupiter, in orbit around Jupiter while the spacecraft maneuvers to rendezvous with Europa, in orbit around Europa, and at and near Europa's surface following a controlled or uncontrolled landing. In this context, it is expected that the requirement will be satisfied by meeting a level of bioload reduction prior to launch that depends on the mission plan, with landers or short-lived orbiters requiring a more stringent level of cleanliness than long-lived orbiters.

A SAMPLE METHODOLOGY

The task group suggests that this analysis consider four main types of organisms:

- *Type A*—Typical, common microorganisms of all types (bacteria, fungi, etc.);
- *Type B*—Spores of microorganisms that are known to be resistant to environmental insults (such as desiccation, heat, and radiation);
- *Type C*—Spores that are especially radiation-resistant; and
- *Type D*—Rare but highly radiation-resistant nonspore microorganisms (e.g., *Deinococcus radiodurans*).

The basis for the above categorization is radiation sensitivity. Although each species will have a somewhat different survival response to ionizing radiation, these are the four general categories that can be readily distinguished by straightforward assay protocols.

Calculation of N_{Xs}, the number of organisms of type X that survive to grow in the europan ocean environment, functionally depends on the initial assayed contamination level (bioload) of the spacecraft, expressed as N_{X0} organisms of type X as follows:

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

In this equation, the variables F_1 through F_7 reflect the various factors that affect the bioload and the survival and growth of organisms for the Europa mission. These factors are explained in detail below.

If the sum of all N_{Xs} is found to be much less than one, it is known by the Poisson statistic that this sum is equal to the probability of any organism being successful. Hence,

P_c = Sum (N_{Xs}) in the limit of a small value (e.g., 10^{-4}).

Microbial Populations On Spacecraft

To begin the calculation, it is important to recognize that different classes of organisms are critical to these calculations, and methods must be devised to estimate their abundances. It is assumed that the spacecraft will be cleaned and/or treated to reduce its bioburden of organisms. As a starting point, the current procedures for cleaning and validating a Mars lander that does not carry life-detection experiments will be assumed. Under these procedures, the lander spacecraft is certified to be carrying a total *available* bioload of not more than 300,000 culturable "spores," where the "spores" are defined to be heat-shock-resistant organisms. However, some portions of a Mars spacecraft are solid materials, encapsulated components, and occluded surfaces, and they are not included in the above levels of bioload because they are not "available" for release to the martian environment. For the sake of argument, however, the task group considered a worst-case scenario, in which the long-term corrosive action of ocean water ultimately liberates *all* organisms, wherever they reside. To adjust the estimate of bioload, typical values for surface and buried microbial density on items manufactured inside and outside of controlled environments (e.g., clean rooms) must be taken into account. These values are given in current planetary protection guidelines.[1]

From a series of many thousands of samplings of the Viking landers and subsequent culture studies, information is available on the types of organisms present under clean room assembly conditions. Chemolithoautotrophic organisms were not specifically tested for because at the time, it was widely expected that organic compounds would be present in the martian soil.

The Viking planetary protection studies characterized only the aerobic, mesophilic organisms that grow on trypticase soy agar (TSA) plates. The ratio of total culturable cells to "spores" was found to be quite variable but typically ranged from 3:1 to 60:1.[2] Under present protocols the spacecraft assay is for heat-shock-resistant organisms, presumed to be spores. To be safe, the task group assumed a value of 50 (see the next paragraph) as the factor by which to multiply the number of heat-shock-resistant organisms measured for a given spacecraft to estimate the actual number of organisms that could grow in culture. This value is significantly higher than the average observed. More than 55 percent of the organisms were gram-positive cocci (*Staphylococcus* and *Micrococcus*), characteristic of organisms found in the human body. *Bacillus* organisms accounted for about 24 percent, the *Corynebacterium-Brevibacterium* group accounted for about 15 percent, and *Actinomycetes* and yeasts constituted the remaining few percent.[3]

In studies done on the Surveyor spacecraft before they were sent to the Moon, aerobes outnumbered anaerobes by 5 to 1, both overall and for just the "spore" fraction. [4] Even though autotrophy was not assayed, a search was made for psychrophilic microorganisms, but none were detected on spacecraft surfaces.[5] The factor-of-50 multiplier (see preceding paragraph) takes into account these additional anaerobic microorganisms, which were not assayed in the Viking studies.

In summary, the number of chemolithotrophic organisms on spacecraft is small compared to the number of more common heterotrophs. Since organic compounds may be present in the putative europan ocean, the task group accepts the nominal spacecraft assays that use TSA as the growth medium and assumes them to be indicative of the overall population of organisms that pose a contamination threat.

Type A organisms are all those organisms that are culturable using the standard TSA plating technique. Type B are those known as "spores" in the standard protocol, as determined by their resistance to heat shock. Type C are a subset of Type B and are resistant to higher radiation doses. Type D are a subset of Type A and are also resistant to high radiation doses. To avoid unnecessarily elaborate testing and analysis procedures, the task group suggests that Types C and D be determined by a simple screening test using exposure to ^{60}Co or by some other well-established procedure for dosing with ionizing radiation. The classification criteria suggested at this time are as follows:

- *Type C*—Organisms with 10 percent or greater survival above 0.8 Mrad; and
- *Type D*—Organisms with 10 percent or greater survival above 4.0 Mrad.

For this example, the task group took a level of 10 times the Mars available "spore" bioburden, or 3×10^6 culturable heat-shock-resistant organisms for the total spacecraft bioload, based on typical spacecraft sizes and

proportions of high-technology components for which cleanliness precautions are taken during manufacture. For an actual spacecraft, this level will be determined by both assays and careful inventory of nonassayable parts and their mode of manufacture.

In this calculation, starting populations for each type were estimated as follows: Type B was 3×10^6 "spores." Type A was estimated as 50 times that of Type B, based on previous experience in spacecraft assembly areas (see discussion above). Since data on the radiation resistance of spacecraft microbes are not available at this time, Type C was arbitrarily taken as 0.1 percent of Type B and Type D as 0.1 percent of Type A. All of these population values should be determined by actual measurements of the spacecraft microorganisms. As will be seen in this and most examples, the Type D organisms are the largest problem, although all four types must be analyzed.

F_1—Total Number of Cells Relative to Cultured Cells

It is now recognized, through the use of molecular probes, that of the organisms in any given ecological environment, laboratory cultivation is successful for only a very small fraction of those present. Thus, unsuccessful laboratory cultivation does not imply that the organisms are not viable. Indeed, only 0.2 percent to 0.3 percent of the organisms found in sediments and soils can be cultured using current techniques.[6,7] In eutrophic samples of activated sludge, the fraction is not so small,[8,9] but in seawater the fraction of successfully cultivated microorganisms is very small.[10,11] Because a significant component of spacecraft contamination is known to come from soil (the other major component comes from organisms associated with the human body), the task group assumed conservatively that laboratory cultivation underestimates actual microbial abundance by a factor of 1,000, for each type of microbial subpopulation.

F_2—Bioburden Reduction Treatment

This factor accounts for any treatment of the entire spacecraft, after assembly, that reduces bioload. For example, the Viking lander spacecraft were heat treated and the value of bioload reduction was specified to be 10^{-4}. Other approaches would be to expose the spacecraft to penetrating ionizing irradiation to destroy all microorganisms, or to chemicals such as hydrogen peroxide or ethylene oxide gas to kill many surface organisms. For this sample calculation, no special treatments are assumed, so no credit for bioload reduction can be taken and this factor must be set to 1.0.

F_3—Cruise Survival Fraction

During the cruise phase of the journey from Earth to Europa, the spacecraft is exposed to the ultrahigh vacuum and ultraviolet irradiation environment of deep space. However, since spacecraft are generally wrapped in opaque thermal protection blankets, it is only these outermost surfaces that are exposed to ultraviolet irradiation.

Bacterial spores are known to be generally resistant to high vacuum, even for long periods of time. For this reason, no credit is taken for this remediating factor for organisms of Type B and C (i.e., $F_3 = 1.0$). Ordinary vegetative cells, Type A, are often susceptible to inactivation by extreme vacuum, so the task group took a value of 0.1 for the survival of these cells. Type D cells are radiation-resistant vegetative microorganisms, with some species being highly resistant to desiccation and others not. Hence, for Type D cells the task group assumed a survival fraction of 0.5.

F_4—Radiation Survival

A mission to Europa might place a spacecraft in Europa orbit for several weeks before its orbit decayed and the spacecraft impacted Europa's surface. During orbit, and later, on the surface, the spacecraft components and any microorganisms on board could be exposed to up to several megarads of radiation from Jupiter's radiation belts. The longer the spacecraft stayed in orbit, the higher the radiation dose it would receive. Once impact occurred, spacecraft debris would be exposed to radiation on the surface at a dose rate of 10 to 100 Mrad per month (see Figure 2.3 in Chapter 2). Notwithstanding the possibility of some radiation shielding on board the spacecraft or of burial in the Europa surface, it is highly probable that many contaminant microorganisms transported to the surface would ultimately accumulate several megarads of radiation damage.

Microorganisms exhibit exponential declines in survival at high doses of ionizing radiation according to the following relationship:

$N = N_0 \exp(-D/D_0)$

where N_0 = the initial cell number, N = the number of survivors that form colonies, D = the radiation dose, and D_0= the D37 dose (at which 37 percent of the population survives). This equation indicates that increasing the dose by a factor of 10 should reduce the survival of microorganisms by a factor of 22,000.

The survival of the bacterium *Escherichia coli* falls to 0.1 percent at 1×10^5 rad;[12] for *B. subtilis,* it falls to 0.1 percent at 6×10^4 to 3×10^5 rad; [13,14] for *Bacillus pumilus,* at 9×10^5 rad;[15] and for *D. radiodurans,* at 1.8×10^6 rad.[16] The physiologic state of the cells, determined by the nutrient conditions at the time of irradiation, is an important factor in determining radiation resistance. Similarly, the presence of oxygen and water greatly potentiates the effect of ionizing radiation on living systems. In general, dry and/or anoxic cells are much more difficult to inactivate than is a fully hydrated cell growing aerobically. Thus, it should be noted that the nutrient conditions of the test subjects in the studies mentioned above were not equivalent.[17,18,19,20] The reported numbers, therefore, are not absolute—they only reflect a trend of resistance.

The radiation sensitivity of all cell types is increased during rapid, exponential growth. In the exponential growth phase, *D. radiodurans*'s viability falls to less than 0.1 percent after a dose of 1 Mrad. By contrast, during the stationary phase (after the growth nutrients have been depleted) this vegetative bacterium is very much more resistant to radiation. Numerous reports show that in the stationary physiologic state, its D37 is 1.75 Mrad (at 5 to 22 °C). At lower temperatures (-70 °C), the D37 of *D. radiodurans* is even more dramatic: 3.0 Mrad.[21] And, remarkably, it was shown that the bacterium can grow at >6,000 rad per hour without any effect on viability.[22] It should be noted that a combination of radiation-resistant and mildly thermophilic characteristics has been identified in two members of the Deinococcal family, *D. geothermalis* and *D. murrayi*.[23]

Table A.1 presents the current best estimates of the radiation sensitivities that should be assumed for the four types of organism when assessing the effect of exposure to radiation in space. When the predicted survival fraction is below 10^{-10}, no lower value is assumed because of the difficulty of verification in practical laboratory experiments. For Type A and Type B organisms, the survival fractions are purely exponential with dose. For Type C and Type D organisms, there is a significant "shoulder" of high survivability until a pure exponential curve comes into play at higher doses.

TABLE A.1 Radiation Sensitivity of Microorganisms

Dose (Mrad)	Type D	Type C	Type B	Type A
0.1	9.90×10^{-1}	9.00×10^{-1}	3.53×10^{-1}	1.15×10^{-2}
0.3	9.50×10^{-1}	8.00×10^{-1}	4.39×10^{-2}	1.53×10^{-6}
1.0	8.00×10^{-1}	3.63×10^{-2}	3.00×10^{-5}	1.00×10^{-10}
4.0	1.00×10^{-1}	2.30×10^{-9}	1.00×10^{-10}	1.00×10^{-10}
6.0	1.00×10^{-3}	1.00×10^{-10}	1.00×10^{-10}	1.00×10^{-10}
7.0	1.00×10^{-5}	1.00×10^{-10}	1.00×10^{-10}	1.00×10^{-10}
8.0	1.00×10^{-8}	1.00×10^{-10}	1.00×10^{-10}	1.00×10^{-10}

F_5—Probability of Landing at an Active Site

Much of the europan surface is considered geologically young, with some parts showing evidence of relatively recent activity. The factor F_5 represents the likelihood of landing at a geologically active site on the europan surface. Landing at such a site could allow geologic activity to transport some or all of the spacecraft to a depth sufficient to shield it from the sterilizing effect of the surface radiation environments and eventually allow it to reach a europan ocean. Since a lethal radiation dose at a depth of 1 meter below the surface is accumulated in 7,000 years (see factor F_6), F_5 is the probability that the spacecraft will land at a site where burial to a depth of significantly more than 1 meter will occur in less than 7,000 years. Extrusive volcanic activity could bury the spacecraft and protect it against the radiation environment, and the spacecraft, or some of its parts, eventually could be carried to a depth where it could interact with a global ocean

Assuming an average age of 50 million years, resurfacing models give the probability of activity at any location as on the order of 10^{-3} in 7,000 years.[24] Nonetheless, highly active areas are of particular concern and may

cover as much as 10 percent of Europa's surface. Thus, the task group assigned the very conservative value of 0.1 to factor F_5, the probability of landing at a site where activity might bury the spacecraft or a significant part of it within 7,000 years, allowing eventual access to a global ocean.

F_6—Burial Fraction

Once a spacecraft is on the europan surface, the probability of an organism reaching the ocean would depend on the lag time until the spacecraft was buried at depths greater than about 10 meters to totally protect it from radiation. It would also depend on the intrinsic shielding within the spacecraft and the extent to which a portion of the spacecraft was buried into the near surface upon coming into contact with it (e.g., in the case of a crash of an orbiter, or a purposeful penetration by a hard lander). The dose profile within the surface ice is a strong function of depth (approximately, inverse square), as shown in Figure 2.3 in Chapter 2. For example, if a portion of the spacecraft is buried to 10 cm, it will take only 90 years to accumulate 7 Mrad of dose, but if it is buried to 1 meter the time to 7 Mrad will be 7,000 years. For this illustrative calculation, 50 percent of the spacecraft was assumed to be protected by being buried in ice to a depth of 1 meter or more.

F_7—Probability That an Organism Survives and Proliferates

It is the consensus of all members of the task group that the likelihood that some terrestrial organism can survive and proliferate in an arbitrary environment on some other body is intrinsically small. However, because such extreme diversity is found among terrestrial microorganisms, this probability cannot be assumed to be zero. As difficult as it is to make such an estimate, the task group hazarded a guess by considering four factors that are pertinent to the survival and growth of any organism in any environment:

- It must survive the physicochemical properties of all environments to which it is exposed on the way to a final environment in which it can prosper;
- The final environment must provide key nutrients;
- A source of energy that the organism can exploit must be available; and
- The organism must be able to grow and reproduce in the final environment in which these nutrients and energy resources are available.

As is indicated in the following considerations, this probability may well be as low as 10^{-6}. Yet, because every spacecraft possesses a certain bioload, the extremely low probability is not per se sufficient to eliminate a need to maintain cleanliness and to control the bioburden. The task group analyzed this probability by breaking it down into four separate components (subfactors 7a, 7b, 7c, and 7d), each of which must be satisfied for an organism to survive and multiply once it reaches the europan ocean.

F_{7a}—Survivability of Exposure Environments

Other than the correlation between radiation and desiccation resistance, very little is known about the survival of terrestrial organisms in combinations of environmental extremes. Given this lack of information, the task group assumes that the ability to survive in one set of physical and chemical conditions does not predispose an ability to survive in other conditions. The factors relevant to survival on Europa include pH, ionic strength, toxic ions, cold temperatures throughout the ocean, and the high pressures at depth. Organisms that do not lyse or become poisoned in this environment and are psychrotolerant and barotolerant could survive in a dormant state as currents move them to different regions of the hydrosphere. For this calculation, 20 percent of organisms are assumed to survive. This conservative value may need to be revised downward if recent suggestions that both hydrogen peroxide and sulfuric acid are relatively abundant in Europa's surface ice are confirmed.[25,26]

F_{7b}—Availability of Nutrients

Elemental nutrients are needed by organisms to synthesize key biomolecules. Especially important are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. Other elements, such as sodium, potassium, chlorine, magnesium, and calcium, are needed to maintain electrolyte balance. Virtually all of these are likely to be available in a salty ocean, in various forms of dissolved compounds or—in some cases—as gases. Trace ions, such as the transition elements needed as enzyme cofactors, are generally present in terrestrial seawater and should be present in a europan ocean. The

balance of ions, or the potential nonavailability of some key resource such as phosphorus or nitrogen, could, however, be limiting. Certain molecular nutrients are required by many organisms. The task group took the probability that the entire suite of needed components are present as 50 percent.

F_{7c}—Suitability of Energy Sources

If life already exists on Europa, primary production by chemoautotrophs might support a food web that produces organics and hence contains heterotrophic populations. The task group made a few a priori estimates of the likelihood that an Earth microbe, either heterotrophic or autotrophic, would grow in a novel chemosphere or the likelihood that it would displace indigenous microbiota in a novel biosphere. This lack of fundamental knowledge requires a conservative approach to europan exploration.

Chemolithotrophic microorganisms include both aerobes and anaerobes. Energy sources for aerobic varieties include hydrogen, ammonia, reduced iron, and sulfur. These serve as electron donors for the respiration of oxygen. Anaerobic chemolithotrophs have the capacity to use these same energy sources when there is an appropriate electron-accepting species other than oxygen, such as nitrate, nitrite, or carbon dioxide. Both anaerobes and aerobes are widespread in soils and are therefore expected to be potential contaminants in assembly areas that have unfiltered conduits to outside air.[27] Since anaerobic chemolithotrophs may be more suited to a europan ocean whether or not indigenous biota are present, the task group discussed only this group in more detail.

Although their requirement for an oxygen-free environment for growth would seem to suggest that anaerobes would be less likely contaminants in an assembly area, it has been well documented in numerous studies that viable anaerobic bacteria are present in oxic, well-drained soils.[28,29] Recoverable anaerobes include non-spore-forming autotrophic types such as methanogens as well as many spore-forming varieties, most notably the acetogens. Both methanogens and acetogens have the capacity to grow on molecular hydrogen and carbon dioxide, using these substrates for carbon and energy, yielding either methane or acetate as the main end product of metabolism. In addition, many acetogens also have the capacity to grow heterotrophically. Sulfate reducers combine sulfate salts and reducing species such as molecular hydrogen as an energy source and produce reduced compounds such as elemental sulfur or hydrogen sulfide.

The relative and absolute abundance of anaerobic species has been estimated for a variety of soils using culture-based methods. The task group emphasizes, as discussed above, that these methods have probably greatly underestimated the abundance of most chemolithotrophic populations.[30] For example, a study of autotrophic H_2/CO_2- consuming methanogens showed that the numbers determined by culturing were about an order of magnitude lower than the numbers estimated by direct microscopic examination.[31] Facultative anaerobes generally constitute about 10 percent of the total aerobic population and are several orders of magnitude more common than the obligate anaerobes. In one study of the soil in a beech forest, the concentration of H_2/CO_2-utilizing anaerobes ranged from approximately 10 to 1,000 cells/g. Since these results are culture-based, they are almost certainly significant underestimates.[32]

The task group also noted that people can harbor significant numbers of autotrophs, including methanogens and acetogens, in their gut.[33] Thus, autotrophic anaerobes are anticipated to be common contaminants in most spacecraft assembly environments.

The probability that for any given assembly of organisms found on a spacecraft there will be a species that is capable of utilizing the exact energy couples available in the europan ocean is, of course, small because of the natural diversity of these populations; this factor is taken as 0.001.

F_{7d}—Suitability for Active Growth

Here the task group considered the two most likely growth environments: the rock-water interface at the bottom of an ocean and the water-ice interface at the top of the ocean. At the rocky boundary, rock-water hydrothermal conditions analogous to Earth's deep-sea hydrothermal vents may occur and produce energy couples that can be exploited. However, because of the extreme depth of Europa's ocean (~80 to 170 km) compared to Earth's, which is only a few kilometers deep, the environment will be at a much higher pressure, even given the lower gravitational attraction of this smaller body. To prosper here, organisms would have to be highly barophilic.

Alternatively, psychrophilic organisms might inhabit the water-ice interface just below the surface ice layer, exploiting the reaction of chemically reduced components in the ocean water with oxidized species from the ice surface or utilizing organic or other compounds in the water itself. Such organisms would have to be highly psychrophilic, however, because of the near-freezing temperatures that would prevail.

For this sample calculation, the task group took the likelihood of a suitable organism to be no more than 1 percent of the organisms that are preadapted to the other environmental factors given previously.

Joint Probability for F_7

The four subfactors that make up F_7 are summarized in Table A.2, along with the calculated joint probability.

TABLE A.2 Basis of F7

Subfactor	Probability
F_{7a} – Survivability of exposure environment	2.0×10^{-1}
F_{7b} – Availability of nutrients	5.0×10^{-1}
F_{7c} – Suitability of energy sources	1.0×10^{-3}
F_{7d} – Suitability for active growth	1.0×10^{-2}
F_7	1.0×10^{-6}

Conclusions from the Illustrative Calculation

The conclusion from this admittedly highly approximate type of calculation are given in Table A.3. They indicate that sterilization of a relatively clean spacecraft by the natural radiation environment can be sufficient to protect the europan ocean environment. The task group notes that the 10^{-4} standard is met if all portions of the spacecraft receive a radiation dose of 7 Mrad. But a 6-Mrad dose would fall far short of being sufficient to achieve the 10^{-4} standard. On the other hand, an 8-Mrad exposure would appear to give extremely favorable results regardless of most other assumptions.

TABLE A.3 Probability of Contamination

	Type D	Type C	Type B	Type A
Number of Culturable Organisms on Spacecraft	1.5×10^5	3.0×10^3	3.0×10^6	1.5×10^8
F_1—Total cells/CFUs	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3
F_2—Bioburden reduction treatment	1.0	1.0	1.0	1.0
F_3—Cruise survival fraction	0.50	1.0	1.0	0.10
F_4—Radiation survival fraction*	1.0×10^{-5}	1.0×10^{-10}	1.0×10^{-10}	1.0×10^{-1}
F_5—Probability of landing at an active site	0.10	0.10	0.10	0.10
F_6—Fraction buried under more than 1 m of ice	0.50	0.50	0.50	0.50
F_7—Probability of survival and proliferation	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
Product of Factors and Organisms	3.8×10^{-5}	1.5×10^{-11}	1.5×10^{-8}	7.5×10^{-8}
Sum	3.8×10^{-5}			

* Values for 7-Mrad dose

Another conclusion reached from this particular sample analysis is that to meet the requirement that $P_c \leq 10^{-4}$, it will be necessary to do at least one of the following:

1. Demonstrate that no Type C or Type D organisms are on the spacecraft; or
2. Demonstrate that the probability of impacting the surface is less than 10^{-4} for the entire time the spacecraft is in the vicinity of Europa (regardless of whether the spacecraft is operational or not); or
3. Show by probabilistic calculations that the 10^{-4} standard can be met through a combination of spacecraft cleaning, selective and/or whole-spacecraft sterilization, and exposure of the spacecraft to the radiation environment at Europa for a long enough period of time to reduce the bioload to the required level (“near sterilization”).

Option 1 is not practical because only a small fraction of microorganisms are culturable. Option 2 may not be possible because of the chaotic perturbations on spacecraft orbits near Europa. Option 3 is achievable using current practices.

Any given mission will need to be individually analyzed. The illustrative calculation presented here indicates that the administration of a dose of at least 7 Mrad to all portions of the spacecraft that have not been previously sterilized and protected from in-flight recontamination is needed to complete sterilization of the most radiation-resistant organisms (Type D). If Type D organisms are present at lower levels than assumed in the example, a lower exposure will be adequate.

To achieve the desired final value, additional approaches could be taken, including the following:

- *Bioburden reduction*—The task group's calculation assumed that no pre-launch sterilization procedures are administered to reduce the bioburden of the spacecraft (i.e., F_2 was assumed to be 1.0). Many technologies could be applied to reduce the bioburden, including heat treatment of the entire spacecraft after cleaning (as was done for Viking), exposure to gaseous chemicals, ultraviolet irradiation of various surfaces, and so forth.
- *Clean-room ecology*—Research to elucidate the populations of organisms in the four categories may show that the assumed number is higher than the number of organisms present in actual assembly conditions for Europa spacecraft.
- *Impact circumstances*—Another factor that could significantly affect the outcome of such calculations are the analyses of how an orbiter spacecraft might impact the surface and to what depths it would be buried. Resurfacing rates at various locations on Europa, and the fractional areas covered, are also highly relevant to this problem.

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B

Glossary

Aerobe —An organism that requires oxygen to grow. See **Anaerobe** .

Anaerobe —An organism with the capacity to grow in the absence of oxygen. See **Aerobe** .

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** .

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

Back contamination —The biological contamination of Earth with material returned from another planetary body. See **Forward contamination** .

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** .

Barophiles —Microorganisms that require high (hundreds of megapascals) hydrostatic pressure for growth.

Bremsstrahlung —Electromagnetic radiation generated when high-energy, charged particles rapidly decelerate during impact with a target.

Cfu (colony-forming unit) —An individual cell that can be grown on a culture plate to form a colony of microorganisms.

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.

Chemosynthesis —The process by which certain organisms use the energy derived from chemical reactions to sustain their metabolism. See **Photosynthesis** .

Cryptoendoliths —Organisms, typically bacteria and lichens, living just below the translucent surfaces of porous rocks found in Antarctica.

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

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** .

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

Gram-positive bacterium —A bacterium that shows a purple color from Gram's stain procedure. The structure of the bacterium's 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.

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

Homoacetogen —A bacterium that produces acetate as an end product and can live as both a chemoautotroph (like methanogens) and a chemoheterotroph.

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.

LET (linear energy transfer) —The energy lost per unit length of path when ionizing radiation passes through a material.

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

Methanogen —A prokaryote that produces methane via the reduction of either carbon monoxide or carbon dioxide in an anerobic environment.

Panspermia —A theory suggesting that life on a given planet may have been seeded by life originating on another planetary body.

PCR (polymerase chain reaction) —A relatively quick and sensitive technique used to detect and generate copies of specific DNA fragments. See **DNA** .

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

Phylogenic —Pertaining to the relationships between different organisms. Such relationships are typically based on comparisons between the DNA sequences of different organisms.

Prokaryotes —Organisms such as the Bacteria and the Archaea whose cells lack a nuclear membrane and other organelles. See **Eukaryote** .

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.

Psychrotrophs — Organisms that have a maximum growth temperature above some 25 to 30 °C and a minimum growth temperature of 5 °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.

RBE (relative biological effectiveness) —A numerical factor used to compare the biological effectiveness of different types of ionizing radiation. It is defined as the inverse of the amount of absorbed radiation of a particular type required to produce a given effect relative to the absorbed dose of a reference radiation (e.g., X rays or gamma rays) required to produce the same effect.

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.

Stationary phase —The period in the growth of a microbial culture after available nutrients have been depleted and population growth ceases.

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

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

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