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Summary of: “Comprehensive Biological Protocol for the Lunar Sample Receiving Laboratory,” Baylor University College of Medicine, NASA CR9-2209, Manned Spacecraft Center, Houston, Texas (1967).

Reason Written: ... a biological quarantine protocol for the safe handling and study of lunar material to be returned to Earth from early Apollo missions ... The goals of the Lunar Receiving Laboratory were multiple and broadly encompass the scientific disciplines of geology, geophysics, chemistry and biology. The purpose of the protocol was to define the biological studies which might reasonably fulfill the goals of the Bioscience Working Group (NASA-SP-88, p. 234, July 1965) “ ... to provide a formal mechanisms for testing appropriate representative lunar samples for the possible presence of agents that might be infectious or toxic to man, animals, and plants. It should be the goal ... to provide safety clearance for lunar samples, if possible, within a period of approximately 30 days.”

The protocol attempted to explore in depth the effect of lunar material upon plants and animal species about which a great deal was already known. The protocol was designed to be flexible and lent itself to easy revision as more information is accumulated concerning the lunar sample and as biological techniques improved during the implementation of the laboratory. The work of the laboratory and protocols was aimed at short-term, time-critical, analytical procedures and identification of whether or not the returned sample constituted a threat to Earth’s biosphere. All other considerations became secondary.

Overview:

The biological protocol has three main elements:

1. crew microbiology (comparisons with pre-flight microbiology profiles and review of alterations in flora following return to Earth) ... conducted under quarantine and limited in duration to the time required to establish the nature of the microbial burden carried by the crew and the assurance of their freedom from communicable disease.
2. *in vitro* attempts to culture microorganisms from the lunar samples;
3. direct challenge of the lunar sample in biological systems

“Acknowledges that ... it will be impossible to tests lunar sample on all but a few Earth species – so portions will be tested in representative members of all major taxa ... utilizes the concept of ‘unity within diversity’ and the careful selection of certain key species to provide a broad-based spectrum for testing purposes.”

Ways that samples may be injurious to organisms from Earth are from inherent toxicity of material or the capability of the material to propagate itself in Earth species.

Toxic materials were classified as follows:

1. Radioactive
2. Unknown inorganic polymer possibly containing silica, boron and other inorganic elements
3. Deleterious low-molecular-weight compounds acting as cellular and metabolic poisons, mutagens, irritants, anti-metabolites or anti-vitamins
4. Unknown metallo-organic compounds, effects on terrestrial organisms unknown

Replicative materials were classified as follows:

1. organisms (viral, bacterial, fungal) taken to the moon and returned in mutated form

2. plant materials of lunar origin capable of reproducing on Earth as autotrophs, heterotrophs in nutrient media – resulting in naturalized forms producing deleterious effects by contact or competition
3. xerophilic life forms of lunar origin using as protoplasmic materials elements found in terrestrial organisms such as carbon, hydrogen, oxygen, sulfur and phosphorous.
4. The existence of living matter on the moon at an organizational level above that of small metazoa or metaphytes ... excluded from consideration because probability considerably less than that for unicellular organisms.

Additional items addressed:

1. Philosophy of testing process itself:
 - Requirement for high professional standards in the conduct of studies
 - NASA should avail itself of technical competence existing in laboratories throughout the country
 - Employ outside consultation at all steps
 - Require a high degree of supervision and insight
 - Laboratory management to utilize fullest sound, competent advice of the academic community and relevant federal agencies (Dept. of the Interior, USDA, US Public Health Service)
2. The nature of the internal controls to be employed
3. The statistical approach to an evaluation of a heterogeneous, unknown mixture whose toxic or microbiological potential is unknown (assumes lunar sample, if it contains microorganisms at all, contains them at very low concentrations. Thus assume at either 'near negligible' or at 'detectable' levels – leading to estimate of high and low quantity of material to be employed in challenges.)

Other important points discussed:

1. Sequence of events in handling samples
2. Collection, transport, receipt, opening as well as mixing, aliquoting and distribution are part of the general protocol
3. Series of challenges to host organisms with both *in vivo* challenges and *in vitro* studies on selected representative plants and animal hosts using classic microbiologic techniques AND parallel studies with both animal and plant cells in tissue culture – all these 'observational' steps to be followed by a secondary *in vivo*-challenge as well as *in vitro* classic microbiological techniques using organic and inorganic media containing such added nutrients as might be suggested by the initial elemental and organic analysis of the lunar sample. *This temporal order of initial, followed by secondary challenges, constitutes the critical part of the microbial protocol* (emphasis added). If replicating forms exist, this sequence offers the greatest promise for their detection.
4. Every system described in the protocol has as an internal control the requirement that direct challenge of *in vivo* systems be conducted with both untreated and sterilized lunar material under absolute double-barrier techniques.
5. Carefully controlled trial runs of all systems should begin fully one year in advance of receipt of the first lunar samples, and 'unknown' terrestrial soil samples should be carried through all systems to insure the technical competence of the laboratory facility.

Overview of Implementation of Quarantine Protocols (Summarized in report by J.H. Allton, 1997 to MELTSWG)

Protocols carried out in Class III biological cabinetry operated under negative pressure and behind the secondary barrier included:

1. Direct observation in which lunar material was examined in native state and via washings and sediments with various optical and electron microscopes up to 1000X magnification
2. Bacteriology/mycology protocols – lunar sample distributed on un-enriched and enriched culture media at temperatures ranging from 4 C to 55 C under cover gases supporting aerobes, microaerobes and anaerobes. Prepared lunar sample was tested to support growth of several pathogenic organisms.
3. Virology and mycoplasma protocols for toxic effects in which tissue cultures of African green monkey (GMK), human embryonic kidney (HEK), and human embryonic lung (HEL) tissues were challenged with lunar sample. For virus isolation, embryonic chicken eggs and 6 tissue cultures (HEK, GMK, HEL, primary duck fibroblast, heteroploid bovine kidney, and heteroploid porcine kidney) were challenged with lunar material. Poikilothermic animals such as trout, minnow, and grunt fin, and 3 mycoplasma media were exposed to lunar material.
4. Mammalian protocols in which mice (180) were injected with lunar material and cultures and tissue samples were taken from sacrificed animals.
5. Avian protocols in which finely powdered suspension injected intraperitoneally into 90 Japanese quail.
6. Invertebrate and fish protocols in which lunar material was added to food for terrestrial and to water for aquatic animals. Test organisms were paramecium, planaria, oyster, cockroach, house fly, wax moth, brown shrimp, killifish, guppy, and minnow.
7. Botany protocols in which assessments were made of lunar sample effects on reproduction and morphology of algae, germination and development of spores and seeds, growth of seedlings, growth and differentiation in tissue cultures. Thirty-five species used including algae, onion, tobacco, radish, spinach, cotton, tomato, potato, wheat, bean, etc. (mostly food crops).
8. Each class of protocol had a decision tree for quarantine testing or sample release recommendations, but all were similar: If any differences between exposed group and control occurred that were not explained as terrestrial contamination, then second order testing was recommended; otherwise release of samples was recommended. No evidence of replicating agents was found in the test systems used, and all samples were released unconditionally.

Summary of: “Orbiting Quarantine Facility (OQF); The Antaeus Report,” Donald L. DeVincenzi and John R. Bagby, Editors, NASA SP-454, NASA, Washington, D.C. (1981).

Charter: A NASA design study was conducted in 1978 to examine the feasibility of designing, constructing, and operating a unique space-based laboratory – one dedicated, at least initially, to the isolation and analysis of potentially hazardous samples returned from Mars. This report does not argue that analysis of Mars samples should be done in space. Rather, it defines the characteristics of an orbiting laboratory should this be an option for active consideration for future MSR studies. Hence, a considerable effort was devoted to development of an appropriate series of tests to be performed on the sample (the quarantine protocol) and to design of the facility in which these tests would be conducted. The 10-week summer study involving 20 scientists and engineers was intended to be an intensive learning experience for the participants.

Background: As a result of the Viking missions to Mars, a great deal of knowledge was gained about the surface features and composition the planet. However, one of the major questions that prompted the mission – Is there life on Mars? – was not conclusively answered. Because of that uncertainty, many scientists believed that the samples should be considered to be potentially hazardous until proven conclusively that they are not. This meant that adequate precautions need to be taken to protect the Earth’s biosphere until the samples are proved safe. Previously, consideration had been given to returning a sterilized sample. Alternatively, it had been suggested that the sample be held under quarantine in a maximum containment facility on Earth, possibly in a remote location, while undergoing analysis. No one had studied a third option, which was to perform hazard analysis of the sample before it was introduced into the terrestrial biosphere. Therefore, this summer study was convened in 1978 to examine the feasibility of receiving and analyzing returned Mars samples in an orbiting quarantine facility.

Summary and Conclusions:

Mission objective: The purpose of the Orbiting Quarantine Facility (OQF) would be to detect the presence of biologically active agents – either life forms or uncontrolled (replicating) toxins – in the sample and to assess their potential impact on terrestrial systems. Only when the sample could be certified safe or controllable would it be transferred to laboratories on Earth for physical analysis.

The particular advantage of an orbiting facility over an Earth-based one is the flexibility it offers in the event that potentially pathogenic agents are present in the sample. With space as a buffer between such organisms and the terrestrial biosphere, the risk of terrestrial contamination is far lower. Complete characterization of the hazard such organisms might represent could thus be carried out without fear of a containment failure and possible contamination of the biosphere. Depending upon the results of testing, the options available for subsequent disposition of the sample would include: 1) unqualified release, 2) sterilization prior to release to Earth laboratories, 3) indefinite retention in orbit for prolonged study, and 4) in one extreme case, boosting the sample-containing facility into a distant orbit. A terrestrial quarantine facility could not offer such margins of security.

Mission scenario: The mission plan calls for the Space Shuttle to deliver the OQF, one or more components at a time, into near Earth orbit, where it will be assembled and manned. While awaiting the arrival of the Mars sample return vehicle (MSRV), the crew will conduct system tests and protocol review. The incoming MSRV, bearing the sample in a sealed canister in its crown, will be inserted into the same orbit in the vicinity of the OQF. An orbiting transfer vehicle comprised of an inertial upper stage engine (IUS) and remote-teleoperated-manipulator system (TELLE) will then link up with the MSRV, extract the sample canister, and deliver it to the OQF. Re-supply of the laboratory, replacement of crewmembers if necessary and eventual transport of the sample and crew to Earth will all be carried out via the Space Shuttle.

Modules: The proposed facility will consist of five Spacelab-derived modular units, each dedicated to a specific function or group of functions. The overall OQF will be free flying and will have a pinwheel configuration, with four of the cylindrical modules connected spoke-fashion to a central hub. Such a

design produces low aerodynamic drag and is easy to assemble; it also allows efficient intermodule movement.

Central to the OQF mission is the Laboratory Module, in which the quarantine testing protocol will be carried out. This unit is equipped with a centrally located containment cabinet system for sample handling and processing. To obtain greater containment reliability than is offered by rubber gloves, specially designed metal bellows manipulative arms will be employed for access to the cabinets. Provision is made to maintain portions of the cabinetry under simulated martian environmental conditions, and a variety of other controlled environments required by the protocol can be produced. Clean air is continuously passed down the face of the cabinets, which are kept under negative pressure to eliminate leakage into the laboratory.

The high-hazard containment facility at the Center for Disease Control (CDC) served as a model for design of many of the physical features and procedures employed in the Laboratory Module. Based on CDC practices, the module itself acts as a barrier to contamination. All equipment and materials leaving the laboratory must be sterilized and packaged in leak-proof containers. Personnel entering or leaving the module must pass through a decontamination area, where they disrobe and take an air shower. The laboratory has independent life support, waste storage, and air filtration systems, and its atmospheric pressure is slightly lower than that of the other modules – all features that ensure effective containment. It is fully equipped for the performance of the quarantine protocol. A variety of microscopes, including scanning electron microscope, are provided. Cameras, spectrophotometers, centrifuge and vacuum devices, autoclaves, refrigerators, and all other necessary laboratory equipment and instruments are present as well.

Four other modules comprise the OQF. The Habitation Module is the crew's living quarters. The OQF's source of power is the Power Module. A general purpose Logistics Module provides storage for supplies and for waste materials generated in the Habitation Module (the Laboratory Module has independent waste storage). A Docking Module, serves as a common interface linking the other four.

Personnel: The crew would probably consist of five members: a commander (an astronaut/engineer) and four scientists (a medical doctor, a geobiologist, a biochemist, and a general biologist). Their tasks would be of two general types: facility operation and maintenance, and laboratory work. The allocation of functions and the scheduling of activities have been carefully worked out for each crewmember.

Experimental protocol: A number of factors impact the experimental design. For example, the protocol must take into account the limited amount of sample available for testing (probably about 100 g). In addition, it must ensure that the untested portion of the sample remains unaltered. It must include a sufficient range of tests to allow biologically active agents to be detected with a high degree of confidence. Equipment and experiments alike must be appropriate for use in the zero-g environment. The potential for human error must be minimal. And there must be enough flexibility designed into the protocol to permit a thorough characterization of life forms that might not closely resemble terrestrial forms.

Preliminary handling: The protocol begins with receipt of the sample canister from the IUS-TELLE. A collapsible structure in the OQF guides the transfer vehicle into position so that a trigger mechanism and clamp can acquire the canister and draw it into the OQF's airlock. The sample canister is punctured with a needle and a sample of the gas within the canister is taken. A mechanism similar to a can opener then removes the bottom of the canister so that further gas sampling and removal of a subsample can take place. The subsample, consisting of approximately 100 g (or 10 percent) of the returned sample, is first analyzed for radioactivity and then transferred by a manipulator to a sample processing unit.

This unit is specially designed to permit the subsample to be manipulated in the absence of gravity, by means of centrifugal force. In the processing unit, the sample is sized and larger particles are viewed under a stereomicroscope to determine whether organisms or fossils are present. The larger-sized material is then evenly ground and the entire subsample is recombined and mixed. This mixture is dispensed to the five testing phases. Of the 100-g subsample, 46 g will be used in the various tests; 54 g

will be held in reserve for possible further series of tests. The remaining 900 g of sample material is stored, unopened, under martian environmental conditions for later delivery to Earth (if approved).

Testing protocol: The five testing phases, and the specific experiments they include, are:

1. Chemical analysis
 - pH, Eh, and conductance tests
 - aqueous extraction/element analysis
 - organic mass spectrometry
 - amino acid analysis
2. Microscopy
 - stereomicroscopic examination
 - scanning electron microscopy
 - light microscope examination
 - ultraviolet microscopy
3. Metabolic testing
 - gas exchange: dry
 - CO₂ fixation: dry and moist
 - enriched O₂ metabolism
 - autoradiography of labeled samples
4. Microbiological culturing
 - growth on solid media
5. Challenge culture

The challenge culture phase involves the introduction of martian soil into cell cultures representing a cross section of terrestrial species. Although a number of organisms have already been tested in zero g to date, additional research is necessary to determine the most appropriate species to include in the challenge system. Such organisms must not only be representative of the Earth's major phyla, but must also have a minimal reaction to zero g.

If results of the preceding series of tests show no evidence of non-terrestrial life forms or replicating toxins, the sample will be approved for delivery to Earth, where more extensive physical, chemical, and biological studies will be undertaken. However, in the event that biological agents have been detected, second order tests would be initiated. The precise character of second-order testing cannot be established in advance. The type of tests would be determined on the basis of characteristics such organisms or toxins might possess.

Protocol planning: The protocol is a complex network of interdependent tests, with many activities being dependent upon the outcome of previous tests. To illustrate the sequence of events in the protocol, a tracking technique known as Graphical Evaluation and Review Technique (GERT) is used. GERT charts present test activities and information flows in their proper sequence, and use GERT 'symbology' to indicate the logic that determines each protocol step. By this means, it is possible to calculate the probabilities associated with different experimental outcomes, and thus to calculate the detection sensitivity of various tests. Detailed GERT charts are presented for each testing phase, along with tables of associated outcome probability analyses.

Conclusion: The facility and the experimental protocol described here offer a strong margin of protection against the possibility that a Mars sample would contain hazardous agents. They also offer a powerful hedge against the unknown, and against the fears that could easily develop if organisms showing signs of pathogenicity were detected in a sample undergoing study in a laboratory on Earth. With such a sample held in orbit, its disposition could be determined on the basis of analysis rather than emotion, and the scientific value of the returned sample could thus be maximized.

Summary of: “Biological Contamination of Mars: Issues and Recommendations,” Task Group on Planetary Protection, (Chair: Kenneth Nealson), Space Studies Board, National Research Council, National Academy Press, Washington, D.C., (1992).

Available online: www.nas.edu/ssb/ssb.html (then select ‘Reports’ and ‘1992’).

Reason Written: In anticipation of planned robotic missions to Mars in the early 1990’s by both US and Russia, NASA requested SSB advice on how to update the nature of planetary protection requirements to reflect changes in the years since the Apollo and Viking missions, and to incorporate new thoughts about life on Mars and the growing environmental awareness of the populace. Recommendations were requested in time for the 1992 COSPAR meeting in order to update international planetary protection policies as needed.

Background: The Task Group focused on making recommendations concerning the protection of Mars from *forward contamination* (i.e., contamination of the martian environment by terrestrial organisms) during upcoming missions. It specifically considered then-current views about the chemical and physical properties of Mars, as well as the potential survival of Earth organisms on Mars, and the approaches to planetary protection used by the U.S. and Russia. In its deliberations, the task group distinguished between missions whose goals included reconnaissance and measurement vs. those that specifically included experiments to detect life.

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

1. Based on current knowledge of conditions on Earth that limit cell growth and on the best estimates of surface conditions on Mars, *the task group concludes that no known terrestrial organisms could grow on the martian surface*. However, this fact does not alter the case as far as contamination of a possible past or extant martian biosphere is concerned. Prudence dictates that bioload reduction on all lander missions to Mars must continue to be seriously addressed. The issue of spacecraft cleanliness is particularly crucial when life-detection experiments are included in the scientific payload.

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

2. Advances in techniques for assessing the existence of microorganisms will have a strong impact both on bioburden assessment procedures and on future life-detection experiments because of their increasingly greater sensitivity and specificity. *The task group strongly recommends that efforts be made to explore current analytical methods for use in bioburden assessment and inventory procedures before spacecraft assembly and launch*. Specific promising methods identified included epifluorescent microscopic techniques for directly counting viable cells, and the polymerase chain reaction which increases detection sensitivity by enzymatically amplifying specific biomarkers of even a single cell to detectable levels.

Recommendations for control of forward contamination:

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

2. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level pre-sterilization procedures – such as clean-room assembly and cleaning of all components – for bioload reduction, but such spacecraft need not be sterilized.
3. The task group emphasizes that the philosophical intent underlying the 1978 report – to protect Mars from terrestrial contamination so as not to jeopardize future experiments aimed at detecting martian life – is still profoundly important.

Additional Recommendations:

1. Research: The task group strongly recommends that a sequence of un-piloted missions to Mars be undertaken well in advance of a piloted mission. With regard to these missions, the task group recommends that a broad spectrum of martian sites be examined, with emphasis on measurements that provide data most likely to contribute to models that provide for a better understanding of the probability of life on Mars and where best to go to find it.
2. Assessment of Spacecraft Bioload: The task group's recommendation to reduce bioload on all spacecraft and to sterilize those spacecraft used in life-detection missions assumes the use of Viking procedures. However, *the task group recommends that the Viking protocols for assessment of spacecraft bioloads be upgraded to include state-of-the-art methods for the determination of bioload.* It is critical that methods for assessing bioload be compatible with methods used to detect life, with methods for both assessment and detection reflecting the same limits and sensitivity. ... modern methods of bioburden assessment should be developed for and applied to spacecraft destined for future Mars missions, especially those carrying in situ extant life-detection experiments. ... the development of the methodology in anticipation of future life-detection missions is absolutely essential.

Other Issues:

1. Piloted Versus Un-piloted Missions: Missions carrying humans to Mars will contaminate the planet. It is therefore critical that every attempt be made to obtain evidence of past and/or present life on Mars well before these missions occur.
2. Societal Issues: A substantial number of active national and international organizations are on the alert for environmental abuse. There is every reason to take seriously the concern (already expressed in some cases) about contamination of Mars and almost certainly about the issue of back contamination of Earth by martian samples. ... *the task group recommends that NASA inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return.*
3. Legal Issues: There are also legal issues that must be addressed, involving international restrictions as well as federal, state, and local statutes that may come into play. There are currently no binding international agreements concerning forward or back contamination. *The task group recommends as essential that efforts be made: 1) to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration, and 2) to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars. Furthermore, the task group recommends that NASA make a strong effort to obtain international agreement for a planetary protection policy.*
4. NASA Planetary Protection Program: Although a planetary protection officer currently exists at NASA, there is no budgeted program (as there was during the Viking Program) to implement needed planetary protection research, a public education program, examination of legal and international issues, and the like. *The task group recommends that NASA redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the recommendations made in this report.*

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

1. Efforts should be made to adopt current molecular analytical methods for use in bioburden assessment and inventory procedures for spacecraft assembly and launch for future missions, and also to develop new methods for the same purposes (Chapters 4 and 5).
2. Landers carrying instrumentation for in situ investigation of extant martian life should be subject to at least Viking-level sterilization procedures. Specific methods for sterilization are to be determined; Viking technology may be adequate, but requirements will undoubtedly be driven by the nature and sensitivity of the particular experiments. The rationale for this requirement is the reduction, to the greatest feasible extent, of contamination by terrestrial organic matter that is deposited at the site by microorganisms or organic residues carried on the spacecraft (Chapter 5).
3. Spacecraft (including orbiters) without biological experiments should be subject to at least Viking-level presterilization procedures – such as clean-room assembly and cleaning of all components – for bioload reduction, but such spacecraft need not be sterilized (Chapter 5).
4. A sequence of unpiloted missions to Mars should be undertaken well in advance of a piloted mission (Chapter 6).
5. A broad spectrum of martian sites should be examined with emphasis on measurements that provide data most likely to contribute to a better understanding of the probability of life on Mars and where best to go to be able to detect it (Chapter 6).
6. The Viking protocols for assessment of spacecraft bioloads should be upgraded to include state-of-the-art methods for the determination of bioload (Chapter 6).
7. NASA should inform the public about current planetary protection plans and provide continuing updates concerning Mars exploration and sample return (Chapter 6).
8. It is essential to assess the legal limits (and implied liabilities) in existing legislation that relates to martian exploration and to pursue the establishment of international standards that will safeguard the scientific integrity of research on Mars (Chapter 6).
9. NASA should make a strong effort to obtain international agreement for a planetary protection policy (Chapter 6).
10. NASA should redefine the responsibilities and authority of its planetary protection officer and provide sufficient resources to carry out the above recommendations (Chapter 6).

Summary of: “Mars Sample Return: Issues and Recommendations,” Task Group on Issues in Sample Return, (Chair: Kenneth Nealson), Space Studies Board, National Research Council, National Academy Press, Washington, D.C., (1997).

Available online: www.nas.edu/ssb/mrsrmenu.html

Reason Written: As stated in NASA Management Instruction 8020.7, the Space Studies Board (SSB) of the National Research Council (NRC) serves as the primary adviser to NASA on planetary protection policy, the purpose of which is to preserve conditions for future biological and organic exploration of planets and other solar system objects and to protect Earth and its biosphere from potential extraterrestrial sources of contamination. In October 1995 NASA requested that the SSB examine and provide advice on planetary protection issues related to possible sample-return missions from Mars and other near-Earth solar system bodies. In response, the Space Studies Board established the Task Group on Issues in Sample Return to address the following concerns:

1. The potential for a living entity to be included in a sample to be returned from another solar system body, in particular Mars;
2. The scientific investigations that should be conducted to reduce uncertainty in the above assessment;
3. The potential for large-scale effects on the environment resulting from the release of any returned entity;
4. The status of technological measures that could be taken on a mission to prevent the unintended release of a returned sample into Earth’s biosphere; and
5. Criteria for controlled distribution of sample material, taking note of the anticipated regulatory framework.

Although focused on sample-return missions from Mars, the recommendations can be generalized to any mission that could return a sample from an extraterrestrial object with a similar potential for harboring life.

Findings:

1. *Although current evidence suggests that the surface of Mars is inimical to life as we know it, there remain plausible scenarios for extant microbial life on Mars – for instance in possible hydrothermal oases or in subsurface regions.*

The surface environment of Mars, from which early samples are most likely to be returned, is highly oxidizing, is exposed to a high flux of ultraviolet radiation, is devoid of organic matter, and is largely devoid of liquid water. It is unlikely that life of any kind, as we currently understand it, either active or dormant, could survive in such an inhospitable environment. If active volcanism, or near-surface liquid water, is discovered on Mars, or if the subsurface environment is found to be considerably less oxidizing and wetter than the surface, the occurrence of extant life on the planet becomes more plausible.

2. *Contamination of Earth by putative martian microorganisms is unlikely to pose a risk of significant ecological impact or other significant harmful effects. The risk is not zero, however.*

In the event that living martian organisms were somehow introduced into Earth’s environment, the likelihood that they could survive and grow and produce harmful effects is judged to be low. Any extant martian microorganisms introduced into Earth’s biosphere would likely be subject to the same physical and chemical constraints on their metabolic processes as are terrestrial organisms. Thus, extraterrestrial organisms would be unlikely to mediate any geochemical reactions that are not already catalyzed by Earth organisms. They would be unlikely to be able to compete successfully with Earth organisms, which are well adapted to their habitats.

Because pathogenesis requires specific adaptations to overcome the extensive defenses possessed by all Earth organisms, virulent extraterrestrial pathogens are unlikely. Subcellular disease agents, such as viruses and prions, are biologically part of their host organisms, and so an extraterrestrial source is extremely unlikely. Conceivably, putative extraterrestrial organisms could be capable of opportunistic infections or toxicity, as are some terrestrial bacteria, but such a risk can be eliminated by standard laboratory control procedures.

The potential for large-scale effects, either through pathogenesis or ecological disruption, is extremely small. Thus, the risks associated with inadvertent introduction of exogenous microbes into the terrestrial environment are judged to be low. However, any assessment of the potential for harmful effects involves many uncertainties, and the risk is not zero.

3. *Uncertainties with regard to the possibility of extant martian life can be reduced through a program of research and exploration that might include data acquisition from orbital platforms, robotic exploration of the surface of Mars, the study of martian meteorites, the study of Mars-like or other extreme environments on Earth, and the study of returned samples. However, each returned sample should be assumed to contain viable exogenous biological entities until proven otherwise.*

The Space Studies Board task group strongly endorses NASA's Exobiological Strategy for Mars Exploration (NASA, 1995). Such an exploration program, while likely to greatly enhance our understanding of Mars and its potential for harboring life, nonetheless is not likely to significantly reduce uncertainty as to whether any particular returned sample might include a viable exogenous biological entity—at least not to the extent that planetary protection measures could be relaxed.

Recommendations – Sample Return and Control:

1. Samples returned from Mars by spacecraft should be contained¹ and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.

While the probability of returning a replicating biological entity in a sample from Mars, especially from sample-return missions that do not specifically target sites identified as possible oases,² is judged to be low and the risk of pathogenic or ecological effects is lower still, the risk is not zero. Therefore, it is reasonable that NASA adopt a prudent approach, erring on the side of caution and safety.

2. If sample containment cannot be verified en route to Earth, the sample, and any spacecraft components that may have been exposed to the sample, should either be sterilized in space or not returned to Earth.

The engineering and design of any sample-return mission should incorporate some means of verifying sample containment during transit and prior to return to Earth. Means should also be available to sterilize the sample, and any spacecraft components that may have been exposed to it, in flight or to prevent their return to Earth in the event that containment cannot be verified.

3. Integrity of containment should be maintained through reentry of the spacecraft and transfer of the sample to an appropriate receiving facility.

The points in a mission where loss of containment is most likely to occur include operations on the martian surface; inter-vehicle transfer of sample material; vehicle reentry, descent, and

¹ The words 'contained' and 'containment' are used herein to indicate physical and biological isolation.

² Locations that exhibit active volcanism or where the presence of liquid water is indicated.

landing; and subsequent transfer of the sample container to a receiving facility. Techniques and protocols that can ensure containment at these vulnerable points should be designed into the mission.

4. Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized.

Returned samples should be considered potentially hazardous until they have been reasonably demonstrated to be non-hazardous. Distribution of unsterilized sample material should occur only after rigorous physical, chemical, and biological analyses confirm that there is no indication of the presence of any exogenous biological entity. If any portion of the sample is removed from containment prior to this determination, it should first be sterilized. The development of effective sterilization techniques that preserve the value of treated material for other (non-biological) types of scientific analysis should be the subject of research by NASA and by the science team associated with the sample-receiving facility.

5. The planetary protection measures adopted for the first Mars sample-return missions should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body.

Samples returned from the martian surface, unless returned from sites specifically targeted as possible oases, are unlikely to harbor life as we know it, and there may be some pressure to reduce planetary protection requirements on subsequent sample-return missions if prior samples are found to be sterile. Presumably, however, subsequent missions will be directed toward locations on Mars where extant life is more plausible, based on data acquired from an integrated exploration program, including prior sample-return missions. Thus, planetary protection measures may become more rather than less critical as the exploration program evolves. At some point it may be reasonable to relax the requirements, but this should only be done after careful scientific review by an independent body.

Recommendation – Sample Evaluation:

1. A research facility for receiving, containing, and processing returned samples should be established as soon as possible once serious planning for a Mars sample-return mission has begun. At a minimum, the facility should be operational at least two years prior to launch. The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization. An advisory panel of scientists should be constituted with oversight responsibilities for the facility.

It was evident from the Apollo experience that the science team, and therefore the lunar receiving facility as a whole, would have been more effective if the team members had had prior experience working together as a group on common problems before receiving lunar samples. During the preliminary study of those samples, loss of containment and compromise of quarantine occurred on several occasions. Some of these occurrences might have been avoided had the science team and the receiving facility been operational well before return of the samples.

To avoid similar problems during the initial investigation of returned martian samples and to provide sufficient time to develop and validate the requisite life detection, containment, and sterilization technologies, the receiving facility and its associated science team should be established well in advance of the launch of any sample-return mission. The facility should include appropriately stringent biological containment capability and be staffed by a broadly multidisciplinary team of scientists. When fully constituted, the science team should strive to include diverse expertise in such areas as effective biological containment, geological and

biological sample processing and curation, microbial paleontology and evolution, field ecology and laboratory culture, cell and molecular biology, organic and light stable isotope geochemistry, petrology, mineralogy, and martian geology.

Recommendations – Program Oversight:

1. A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample-return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least one year prior to the establishment of the sample-receiving facility (at least three years prior to launch).

... to coordinate regulatory and other oversight responsibilities, NASA should establish a panel analogous to the Interagency Committee on Back Contamination that coordinated regulatory and oversight activities during the lunar sample-return missions. To be effective, planetary protection measures should be integrated into the engineering and design of any sample-return mission, and, for an oversight panel to be in a position to coordinate the implementation of planetary protection requirements, it should be established as soon as serious planning for a Mars sample-return mission has begun. For the panel to be able to review and approve any plans for a Mars sample-receiving facility, the panel should be in place at least one year before the sample-receiving facility is established.

2. An administrative structure should be established within NASA to verify and certify adherence to planetary protection requirements at each critical stage of a sample-return mission, including launch, reentry, and sample distribution.

An internal administrative structure, with clearly defined lines of authority, is required to verify and certify adherence to planetary protection requirements at each critical stage of a sample-return mission, including launch, reentry, and sample distribution. The certification should be sequential. That is, the mission should not be allowed to proceed to the next stage until planetary protection requirements for that stage and each preceding stage have been met. For example, reentry should not be authorized unless containment has been verified or the material to be returned has been sterilized. The required internal structure is already partly in place at NASA, but the lines of authority should be more clearly specified and a certification process should be implemented for each mission stage.

3. Recommendation: Throughout any sample-return program, the public should be openly informed of plans, activities, results, and associated issues.

In light of the public's past response to other controversies involving science and technology, it is possible that environmental and quality-of-life issues will be raised in the context of a Mars sample-return mission. If so, it is likely that the adequacy of NASA's planetary protection measures will be questioned in depth. The most effective strategy for allaying fear and distrust is to inform early and often as the program unfolds. Acknowledging the public's legitimate interest in planetary protection issues, and thereby keeping the public fully informed throughout the decision-making process related to sample return and handling, will go a long way toward addressing the public's concerns.

Summary of: “Mars Sample Quarantine Protocol Workshop,” D.L. DeVincenzi, J. Bagby, M. Race, and J.D. Rummel, NASA CP-1999-208772, NASA Ames Research Center, Moffett Field, California, (1999).

Reason Written: In 1996, several NASA-sponsored studies were underway to look at various aspects of a Mars Sample Return (MSR) mission. One of these studies by the Mars Exploration Long Term Science Working Group (MELTSWG) determined the need for additional study of five specific areas related to Planetary Protection (PP). One of the priority areas identified was the need to develop guidelines for return sample containment and quarantine analysis. In response to this need, the Mars Sample Quarantine Protocol Workshop was convened in June, 1997 to deal with three specific aspects of the initial handling of a returned Mars sample:

- 1) biocontainment, to prevent uncontrolled release of sample material into the terrestrial environment; 2) life detection, to examine the sample for evidence of live organisms; and
- 3) biohazard testing, to determine if the sample poses any threat to terrestrial life forms and the Earth's biosphere.

Background: In order to constrain the scope of the Workshop, several starting assumptions were given: 1) The Mars Sample Return mission (MSR) will be launched in the 2005 opportunity; 2) the mission will return samples from biologically interesting sites based on data returned from missions in 1996, '98, '01, and '03; 3) in a nominal mission, the sample will not be sterilized prior to return to Earth; 4) the amount of sample available for quarantine tests will be a small fraction of the total amount returned; and 5) biocontainment of the unsterilized sample will be maintained until quarantine testing for biohazards is accomplished.

Findings – Containment: The Containment Subgroup discussed the development of recommendations that might be adopted by NASA for the safely controlled management of a Mars sample while a quarantine protocol is executed. Containment was defined as: “a system of protection of: 1) the Earth's biosphere from release of ‘biological entities’ of martian origin, and 2) the integrity of the sample.”

Recommendations – Containment:

Sample Return Canister: The entire system of containment – from Mars to Earth – must prevent the escape of potentially hazardous material. This means special design considerations for the canister and planning for Earth return procedures. Specific recommendations include:

1. Decontamination of the exterior of the canister that contacts the martian surface;
2. Contingencies for non-nominal events (ex. initial trajectory of Earth return vehicle biased to miss Earth; indicator system to monitor for breach of containment en route; on board system for sterilization in case of an in flight breach in containment; provisions to determine if a breach occurs during a hard impact at the landing site, and suitable sterilization for that event.)

Upon recovery of the canister and reconfirmation of proper containment, the canister must be transported to a quarantine facility in a container meeting regulatory requirements for safe transport of potentially hazardous biological material. Precautions for handling the sample return canister should include provisions for protective garments for the recovery crew and coordination with appropriate regulatory agencies such as USDA-APHIS and EPA.

Mars Receiving Laboratory (MRL): The unknown nature of any possible hazardous material in the sample warrants the use of the most stringent containment presently afforded to the most hazardous biological entities known on Earth; that is, a Biosafety Level 4 (BSL-4) operation. Appropriate containment is attained through the application of primary and secondary containment principles:

1. Primary containment will be provided by utilizing Class III biosafety cabinets – comprised of glove boxes connected in sequence with sealable doors between cabinets and maintained under negative pressure.
2. Secondary containment will be provided by the building: a ‘high-end’ BLS-3 structure which is sealed and maintained under negative pressure, with high efficiency particulate air (HEPA)

filtered exhaust air, sterilized waste water, and with provision for personnel showers and appropriate use of disinfectants.

While biological safety and physical security must be the prime considerations in the design of a Mars receiving facility, there could be alternative approaches to accomplish the needed containment besides a dedicated new facility. One such alternative includes providing a small MRL facility beside an existing approved BSL-4 laboratory (e.g. USAMRIID at Fort Detrick, Maryland, or CDC in Atlanta, Georgia) This would offer flexibility, availability of trained professionals and support staff, and possible simplification of the permit and approval process. Disadvantages include the possible reduction in control of samples by NASA while in the hands of another agency, and background organic residues and contamination that could interfere with sample interpretation. Existing policy for the transport and receipt of potentially hazardous agents requires CDC review of the facility, thereby providing an additional check on safety. Whatever alternative is selected, at least five years must be allowed for the construction and certification of such a highly technical facility and for the training of professional and support staff. Training periods are required for qualified personnel to become familiar with the new facility so their operations are safe, efficient, and accurate.

3. Glove Box System: Glove boxes can be flexibly designed to include any laboratory equipment required by the protocols. Operational parts of equipment can be housed within the primary containment glove boxes, with electronics, control panels, etc. located outside the primary containment barrier. The report provides preliminary details on the need for careful planning of the sequence of steps for handling and opening the sample canister in the cabinet lines to avoid contamination on contained samples.

Containment Research and Technology Needs: Specific research areas recommended to accomplish successful containment both in transit and in the laboratory include:

1. Challenge tests of HEPA filtration system should be undertaken using carbon-bearing particles from 10 nm to 100 nm in size.
2. Research should be conducted to choose appropriate isotopes and particle sizes for use in flight verification and testing of canister seals (e.g., carbon compounds, radioactive-tagged particles).
3. Select an appropriate indicator for canister seal integrity upon recovery
4. Design effective processes to clean containment area of terrestrial biological entities and organics to avoid confusion during observations of the Mars samples.
5. Systems must be developed and tested to maintain sample integrity when obtaining aliquots of material for quarantine testing
6. Design research to provide a system for needle puncture of the 'head space' through a vacuum-sealed line; HEPA filters could be incorporated.
7. Determine the suitable sterilization methods for the Mars sample.

Findings – Life Detection: The Life Detection Subgroup was assigned the task to develop a series of tests (a protocol) to detect the presence of live organisms, or of materials that have been derived from live organisms, in samples of material returned from Mars. The group first considered the likely aspects of viable organisms that might be detected and then determined the philosophy that should guide the life detection protocol, which in turn would dictate the sequence, techniques and handling requirements for the protocol. The subgroup also made recommendations on research needed to refine the eventual protocols.

The philosophy espoused by the subgroup aimed not only at detecting life, but distinguishing between potential martian life forms and terrestrial contamination. In particular: 1) there must be multiple lines of evidence to support an hypothesis that detected life is of martian origin, and 2) it is essential to understand the geological and potential ecological context of a sample in order to understand the nature of life that might be detected in the samples. A strong quality assurance and quality control (QA/QC)

program was deemed essential, involving the use of chemical tracers in order to correlate the 'detected' material/organism(s) with the phase of the mission in which material was obtained.

In order to establish the appropriate context for life detection in a sample, a preliminary analysis of the sample was recommended to: 1) characterize the bulk mineralogy of the sample, 2) establish its elemental composition, 3) inventory the volatile and organic materials it may contain, 4) measure the redox couples present in the sample material, and 5) obtain a microscopic characterization of the sample surface and interior. As long as an adequate sterilization method could be defined which would not affect the results of the analysis, the Subgroup felt most of these analyses would not require the sample to be held in biological containment.

Recommendations – Life Detection:

The Life Detection Subgroup prioritized three basic methods for accomplishing life detection:

1. Organic chemical analysis and detection including search for functional groups containing reduced carbon, sulfur or nitrogen; analysis of possible kerogen materials for stable isotope abundances; detection of amino acids or possible proteins; analysis for amphiphiles in the form of fatty acids, hopanes, etc; a search for carbohydrates, nucleic acid bases, and related compounds (e.g., DNA, RNA, PNA, etc.); and potential detection of integrated cell walls or cell wall components such as lipopolysaccharides. Assuming current improvements in available technologies, it was felt that cellular life could be detected routinely at the level of 10-100 cells in a sample and as little as one cell in a 100 g sample.
2. Light and/or electron microscopy to detect morphological indications of life, along with the trace mineralogy of the sample. Coupled with staining methods to reveal chemical evidence of life in conjunction with morphological methods, light microscopy was seen as having advantages over electron microscopy in terms of sample preparation, handling and real-time testing. Electron microscopy, particularly ion-probe techniques, can provide critical composition information about samples. The issue of what constitutes a 'representative' sample will need to be defined.
3. Culturing of martian materials and/or living organisms: Although it will be difficult to generalize for putative martian organisms, cultivation as a life detection approach was recommended because of the potential to amplify the presence of life in a sample, to discriminate between a viable organism and materials that were once associated with biology (but not now alive), and to provide a natural link to hazard detection analyses. Attempted cultivation techniques should include not only conditions commensurate with the environment from which samples were obtained, but also the use of multiple media and carbon sources under both aerobic and anaerobic conditions, using both intact samples and processed sample materials. Given the low culturability of environmental microbes from Earth (~1%), culturability is of secondary or tertiary priority for life detection.

The Life Detection Protocol should be an integrated facet of the comprehensive analysis of samples for atmospheric, geophysical, and exobiological purposes. A comprehensive process for sample analysis and life detection was outlined which includes detailed comments about particular steps in the process such as the sample container, sample receiving, sample separation, microscopic/mineralogical/geochemical survey, life detection microscopy, and chemical analyses for signs of life. The Life Detection Subgroup recommended that the following considerations form the basic concept of chemical analysis techniques in life detection:

1. Seek functional groups important for energy transfer rather than live biomass
2. Seek to identify accumulated biomass-type molecules and cellular components rather than cells or single living entities
3. Use more sensitive and less selective detectors for the first sample screening procedure. Rather than employing the selectivity of GC-MS or KC-MS as the first step, use highly sensitive infrared micro-calorimetric or lab-on-a-chip technology to provide high sensitivity detection of functional groups.

4. Integrate remnant parts as a preliminary indication of possible extant life (the amount of functional groups remaining from remnant parts often exceeds the live biomass in samples on Earth.)
5. It may not be possible to rely on DNR, RNA, proteins or even carbon-based molecular backbones as indicators because extraterrestrial life may be markedly different in detail from life on Earth. Focus initial screening efforts on amine and carboxyl functional groups to detect signs of life based on any backbone, C, N, P, S or Si. Comparison of stable isotopic signatures of non-life-like compounds (e.g., PAHs) and life-like compounds may provide additional information on the potential existence of life on Mars.

Life Detection Research and Technology Needs: NASA must begin to incorporate life detection technologies into planning and anticipated sample receiving activities for MSR. In particular, a plan must be developed for the acquisition and operation of appropriate instrumentation within the sample handling facility, and appropriate sterilization protocols and methods must be developed to prepare samples for distribution to the wider scientific community.

Findings – Biohazard Testing: The Biohazard Testing Subgroup was assigned the task of developing an up-to-date methodology to determine if returned martian sample materials are hazardous, regardless of whether life or biological entities are detected. The Subgroup proposed a tiered or stepwise approach to testing based heavily on protocols used by research and agencies for a wide range of biological agents. These tests would: 1) focus on a broad range of biohazards, 2) screen for indication of biological activity or disruption thereof, and 3) incorporate systematic feedback as data are gathered from the life detection studies, chemical analyses, and biohazard tests themselves. Emphasis was placed on hazards posed by organisms that replicate because of their potential for large scale negative impacts on Earth's ecosystems.

Two priority biohazard concerns were addressed: 1) pathogenicity, and 2) ecological disruption. (Chemical toxicity was not considered a significant biohazard or global threat since toxic materials will not replicate and spread, and since proper laboratory protocols will protect those who work with the samples). Detailed information and discussion about various tests are provided in the appendix of the report. In general, the subgroup recommended the following:

Recommendations – Biohazard Testing:

Pathogenicity: Regardless of the outcome of preliminary life detection tests or chemical analyses, it will be prudent to screen samples for two types of pathogenicity – toxic and infectious – using tests specifically designed to detect biological activity or disruptions. *In vitro* methods are considered superior to whole organism tests for preliminary biohazard screening because of their sensitivity, simplicity and speed, as well as their widespread use, acceptance and interpretation. By selecting a suitably diverse range of *in vitro* tests and conditions, it will be possible to screen for biologically important outcomes that might be indicative of biohazards in a wide range of representative species and taxonomic groups. It would be advisable to include a range of *in vitro* tests that are routinely used by agencies and researchers when scanning for pathogenesis. In addition, the inclusion of two additional types of tests – a series of laboratory mice injection studies (because of their extensive use for pathogenicity and biohazard testing) and a series of tests using Tetrahymena (as a model for metazoan biochemistry) – were discussed. A recommended battery of tests for detection indication of potential pathogenicity in the sample might include:

1. diverse microbial media that use varied laboratory initial conditions
2. selected tissue cultures and cell lines from mammalian organ systems, fish and insects
3. embryonating chicken eggs
4. mouse injection studies
5. Tetrahymena (protozoans)
6. Plant tissue cultures (wheat, rice, potato).

Ecological Disruption: In the event of inadvertent introduction to the Earth's biosphere of putative martian microbes, there would be little threat of widespread ecological disruption based on our comparative knowledge of martian and Earth conditions and our knowledge about microbial potential on Earth. Nevertheless, since the risk of potentially harmful effects is not zero, it will be prudent to screen for the ability of the returned sample to disrupt microbial ecosystems. Although such tests are not routinely done, it would be advisable to design and conduct suitable microcosm tests to screen for potential ecosystem effects or disruption in biogeochemical cycles. Two types of microcosm tests are recommended, the first designed to assay for disruptions of important representative microbial systems upon addition of martian material, and the second to determine if any undetected biological entities can grow or propagate in selected sterilized microcosm of representative terrestrial ecosystems

Criteria for Distribution of Martian Samples: The Biohazard Testing Subgroup considered the many possible interpretations of data for the proposed battery of life detection and biohazard tests and developed a table providing an overview of various combinations of findings (Table 1 in report). In general, if any life forms are detected, even if preliminary test suggest they do not pose a biohazard, the Subgroup advised continued strict containment, rather than controlled distribution, at least initially. Strict containment should be maintained in light of any positive test results until findings are verified and/or a scientific panel provides further guidance on subsequent handling. All verification testing should use only *in vitro* tests under BSL-4 containment. No consensus was reached on what containment/ release recommendations should be made if all life detection and biohazard tests are negative. Additional discussion will be needed to translate the various test outcomes into specific recommendations for release of unsterilized materials from containment.

Biohazard Research and Technology Needs: Specific recommendations for R&D related to biohazard testing were identified in the following areas:

1. Validation of methodological approach (cell and tissue test rather than whole organisms studies; pre-testing of efficacy; techniques for characterizing any isolated or suspected life forms etc.)
2. Microcosm Research (development, effectiveness; predictive value; non-destructive, long-term observation and sampling, etc.)
3. Representative samples, controls and replicates
4. Other operational issues (training and monitoring programs for lab personnel; management of lab operations and facilities; issues related to limited quantities of material, sample allocation, research access, and evaluation of research proposals).

Summary of: “Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies,” Task Group on Sample Return from Small Solar System Bodies, (Chair: Leslie Orgel), Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1998).

Available online: www.nas.edu/ssb/ssb.html (click on ‘Reports’ and ‘1998’).

Reason Written: With the advent of possible sample return missions from multiple planetary bodies, NASA asked the Space Studies Board (SSB) of the National Research Council (NRC) in 1997 to assess the potential for a living entity to be contained in or on samples returned from planetary satellites and other small solar system bodies such as asteroids and comets. The Task Group on Sample Return from Small Solar System Bodies was asked to build on and extend earlier SSB studies on Mars (1992 forward contamination report and 1997 sample return report) and address the following specific tasks:

1. Assess the potential for a living entity to be contained in or on samples returned from planetary satellites or primitive solar system bodies, such as asteroids, comets, and meteoroids;
2. Identify detectable differences among small solar system bodies that would affect the above assessment;
3. Identify scientific investigations that need to be conducted to reduce the uncertainty in the above assessment; and
4. Assess the potential risk posed by samples returned directly to Earth from spaceflight missions, as compared to the natural influx of material that enters Earth's atmosphere as interplanetary dust particles, meteorites, and other small impactors.

Background and Study Approach:

Because there is no direct evidence that a living entity evolved or exists on any small solar system body, the task group examined indirect evidence based on data from Earth, meteorites, and the Moon and on astronomical observations of distant objects in an effort to assess whether NASA needs to treat samples returned from small solar system bodies differently from samples returned from Mars. To identify the requirements for the origin and survival of living organisms, the task group examined contemporary views on the range of conditions under which life can originate, the conditions required for the preservation of metabolically active organisms in terrestrial environments, and the somewhat different conditions needed to preserve living organisms in a dormant form. Based on this analysis, the task group identified six parameters (liquid water, energy sources, organic compounds, temperature, radiation intensity, and natural influx to Earth) as relevant to its assessment and formulated the following six questions to help determine how returned samples should be handled:

1. Does the preponderance of scientific evidence indicate that there was never liquid water in or on the target body?
2. Does the preponderance of scientific evidence indicate that metabolically useful energy sources were never present?
3. Does the preponderance of scientific evidence indicate that there was never sufficient organic matter (or CO₂ or carbonates *and* an appropriate source of reducing equivalents) in or on the target body to support life?
4. Does the preponderance of scientific evidence indicate that subsequent to the disappearance of liquid water, the target body has been subjected to extreme temperatures (i.e., >160 C)?
5. Does the preponderance of scientific evidence indicate that there is or was sufficient radiation for biological sterilization of terrestrial life forms?
6. Does the preponderance of scientific evidence indicate that there has been a natural influx to Earth, e.g., via meteorites, of material equivalent to a sample returned from the target body?

In applying the questions, the task group drew on existing data on the origin, composition, and environmental conditions (past and present) of each small body or planetary satellite examined and then determined whether the quality and weight of the evidence were convincing enough to allow making judgments and deriving findings. The answers to the questions, taken together, were used to reach a considered conclusion that the potential for a living entity to be in or on a returned sample was either 'negligible' or 'not negligible.' Because of the incomplete current state of knowledge about small solar system bodies, there are no definitive answers to the questions, and so all judgments regarding biological potential are qualitative (not quantitative).

The questions allow for a conservative, case-by-case approach to assessing whether or not special physical and biological isolation and handling of returned samples (containment) would be warranted, taking into account information about the different small bodies, natural influx to Earth of material from small bodies, and the possible nature of putative extraterrestrial life. An answer of 'yes' to any question argues against the need for special containment beyond what is needed for scientific purposes. For containment procedures to be necessary, an answer of 'no' needs to be returned to all six questions. For such samples, strict containment and handling would be required (similar to the Mars sample return handling recommended by SSB in the 1997 report).

The task group chose to consider only two possible alternatives for containment and handling of samples returned from small solar system bodies, either: 1) strict containment and handling of returned samples as outlined in the Mars report ([NRC 1997](#)), or 2) no special containment beyond what is needed for scientific purposes. The task group ruled out intermediate or compromise procedures involving partial containment.

Findings:

Planetary Satellites: Satellites are natural consequences of planetary formation processes. The task group considered the possibility of sample return from the major satellites of the innermost planets including the satellite of Earth (the Moon), satellites of Mars (Phobos and Deimos), and selected satellites of Jupiter (Io, Europa, Ganymede, and Callisto). The potential for a living entity to be present in samples returned from the Moon and Io is negligible. The potential for a living entity to be present in samples returned from Phobos, Deimos, and Callisto is extremely low, but the task group could not conclude that it is necessarily zero. Importantly, the task group found that there is a significant potential for a living entity to be present in samples returned from Europa and Ganymede.

Asteroids: Asteroids are the remnants of planetesimals – small primordial bodies from which the planets accumulated. Common asteroid types include undifferentiated, primitive types (C-, B-, and G-types); undifferentiated metamorphosed types (Q- and S-types [ordinary chondrites]); and differentiated types (M-, V-, J-, A-, S- [stony irons], and E-types). Other types of asteroids have been defined, including the common P- and D-types in the outer parts of the asteroid belt, but little is known about their composition and origin. Others are subdivisions of the types listed above, whereas still others are rare, new types, generally seen only among the population of very small asteroids. For undifferentiated, primitive (C-type) asteroids, the potential for a living entity to be contained in returned samples is extremely low, but the task group could not conclude that it is necessarily zero. Because of a fundamental lack of information about P- and D-type asteroids, the potential for a living entity to be present in returned samples cannot be determined and, therefore, was considered conservatively by the task group as possible at this time. For all C-type asteroids, undifferentiated metamorphosed asteroids, and differentiated asteroids, the potential for a living entity to be present in returned samples is extremely low, but the task group could not conclude that it is necessarily zero.

Comets: Comets are believed to have formed in the protoplanetary disk, at distances from the Sun ranging from the distance of proto-Jupiter to far beyond the distance of proto-Neptune. It is unlikely that a living entity could exist on comets, but the possibility cannot be completely ruled out except in a few cases, such as in the outer layers of Oort Cloud comets entering the solar system for the first time. Thus,

the potential for a living entity to be present in returned samples from all comets was considered by the task group to be extremely low, but the task group could not conclude that it is necessarily zero.

Cosmic Dust: Because interplanetary dust particles (IDPs) are derived from a variety of sources, including interstellar grains and debris from comets, asteroids, and possibly planetary satellites, IDPs cannot be viewed as a distinct target body. As a result, the assessment approach used in this study does not lend itself readily to IDPs. Instead, the task group considered the potential source(s) of any IDPs that might be returned in samples. For the purposes of this study, IDPs are viewed as originating from either a single identifiable parent body or multiple sources. Particles collected near a particular solar system body are viewed as originating from that body, possibly including grains recently released from that body. Thus, the potential for a living entity to be present in returned samples, and the associated containment requirements, will be the same as those for the parent body. On the other hand, IDPs collected in the interplanetary medium may represent a mixture of dust originating from many parent bodies. Because IDPs in the interstellar medium are exposed to sterilizing doses of radiation, the potential for IDPs to contain viable organisms or a living entity is negligible.

Conclusions and Recommendations:

Table ES.1 summarizes the task group's assessment of the level of containment and handling warranted for samples returned from the planetary satellites and small solar system bodies examined in this study. Box ES.1 summarizes the requirements that apply to samples for which strict containment and handling are advisable. It is important to note that the task group's recommended approach is provided only as a guide and not as an inflexible protocol for determining whether containment is required. The final decision must be based on the best judgment of the decision makers at the time and, when possible, on experience with samples returned previously from the target bodies.

Containment of Returned Samples:

On the basis of available information about the Moon, Io, dynamically new comets (specifically the outer 10 meters), and interplanetary dust particles (sampled from the interplanetary medium, sampled near the Moon or Io, or sampled in a way that would result in exposure to extreme temperatures), the task group concluded with a high degree of confidence that no special containment is warranted for samples returned from those bodies beyond what is needed for scientific purposes.

Recommendations:

1. Samples returned from the Moon, Io, the outer 10 meters of dynamically new comets, and interplanetary dust particles (from the interplanetary medium, near the Moon, Io, or dynamically new comets), or sampled in a way that would result in exposure to extreme temperatures (e.g., spike heated), should not be contained or handled in a special way beyond what is needed for scientific purposes.

For samples returned from Phobos and Deimos, Callisto, C-type asteroids, undifferentiated metamorphosed asteroids, differentiated asteroids, and comets other than dynamically new comets, the potential for a living entity in or on a returned sample is extremely low, but the task group could not conclude that it is zero. Based on the best available data at the time of this study, the task group concluded that containment is not warranted for samples returned from these bodies or from interplanetary dust particles collected near these bodies. However, this conclusion is less firm than the conclusion for the Moon and Io and should be reexamined at the time of mission planning on a case-by-case basis.

2. For samples returned from Phobos and Deimos, Callisto, C-type asteroids, undifferentiated metamorphosed asteroids, differentiated asteroids, comets other than dynamically new ones, and interplanetary dust particles sampled near these bodies, a conservative, case-by-case approach should be used to assess the containment and handling requirements. NASA should consult with or establish an advisory committee with expertise in the planetary and biological sciences

relevant to such an assessment. The goal of such an assessment should be to use any new, relevant data to evaluate whether containment is still warranted. This assessment should take into account all available information about the target body, the natural influx to Earth of relevant materials, and the likely nature of any putative living entities. Such an advisory committee should include both NASA and non-NASA experts and should be established as early in the mission planning process as possible.

For samples returned from Europa and Ganymede, the task group concluded that strict containment and handling requirements are warranted. Because the knowledge base for P- and D-type asteroids is highly speculative, the task group concluded conservatively that strict containment and handling requirements are warranted at this time. Strict containment and handling requirements are also warranted for interplanetary dust particles collected near these bodies unless they are sampled in a way that would result in exposure to extreme temperatures, e.g., spike heated.

3. Based on currently available information, samples returned from Europa, Ganymede, P- and D-type asteroids, and interplanetary dust particles sampled near these bodies should be contained and handled similarly to samples returned from Mars ([NRC 1997](#)). Interplanetary dust particles sampled in a way that would result in exposure to extreme temperatures, e.g., spike heated, should not be contained or handled in a special way beyond what is needed for scientific purposes.

Handling of Returned Samples:

For samples that are returned from planetary satellites and small solar system bodies and that warrant containment, the concerns about biohazards or large-scale adverse effects on Earth are similar to those identified earlier for Mars ([NRC 1997](#)). The task group concluded that the risks of pathogenicity from putative life forms are extremely low, because it is highly unlikely that extraterrestrial organisms could have evolved pathogenic traits in the absence of host organisms. However, because there are examples of opportunistic pathogens from terrestrial and aquatic environments that have not co-evolved with their hosts, the risk cannot be described as zero. The recommendations on containment and handling in the Mars report ([NRC 1997](#)) represent a strong basic framework for addressing potential risks associated with returned samples warranting containment.

The microbial species composition of most anaerobic environments on Earth is not known, and consequently it is also not known how the species composition of these anaerobic microbial communities might change over time, what environmental factors might influence these changes, or what the incidence of and successful colonization by new species of microorganisms in these habitats might be. Accordingly, the task group concluded that although there is a low likelihood of a viable anaerobic microorganism surviving transport through space and finding a suitable anaerobic habitat on Earth, growth in a suitable habitat if found might be possible. This conclusion is necessary because of the current lack of information about anaerobic environments on Earth that may be analogous to environments on other solar bodies, and the likelihood that the metabolic properties of such an extraterrestrial anaerobe would resemble an Earth anaerobe from a similar environment.

For overall evaluation of returned samples that warrant containment, it will be necessary to apply a comprehensive battery of tests combining both life-detection studies and biohazard screening.

Recommendations:

1. Returned samples judged to warrant containment should be quarantined and screened thoroughly for indications of a potential for pathogenicity and ecological disruption, even though the likelihood of adverse biological effects from returned extraterrestrial samples is very low.
2. NASA should consult with or establish an advisory committee of experts from the scientific community when developing protocols and methods to examine returned samples for indicators of past or present extraterrestrial life forms.

3. The planetary protection measures adopted for the first sample return mission to a small body whose samples warrant special handling and containment should not be relaxed for subsequent missions without a thorough scientific review and concurrence by an appropriate independent body.

TABLE ES.1: Summary of Currently Recommended Approach to Handling Samples Returned from Planetary Satellites and Small Solar System Bodies Assessed by the Task Group on Sample Return from Small Solar System Bodies

I No Special Containment and Handling Warranted Beyond What Is Needed for Scientific Purposes		II Strict Containment and Handling Warranted
Ia <i>High Degree of Confidence</i>	Ib <i>Lesser Degree of Confidence^a</i>	
The Moon Io Dynamically new comets ^b Interplanetary dust particles ^c	Phobos Deimos Callisto C-type asteroids Undifferentiated metamorphosed asteroids Differentiated asteroids All other comets Interplanetary dust particles ^e	Europa Ganymede P-type asteroids D-type asteroids Interplanetary dust particles ^d

^aSubcolumn Ib lists those bodies for which confidence in the recommended approach is still high but for which there is insufficient information at present to express it absolutely. This lesser degree of confidence does not mean that containment is warranted for those bodies; rather, it means that continued scrutiny of the issue is warranted for the listed bodies as new data become available. The validity of the task group's conclusion that containment is not warranted for the bodies listed in Ib should be evaluated, on a case-by-case basis, by an appropriately constituted advisory committee in light of the data available at the time that a sample return mission to the body is planned.

^bSamples from the outer 10 meters of dynamically new comets.

^cInterplanetary dust particles sampled from the interplanetary medium and from the parent bodies listed in subcolumn Ia.

^dInterplanetary dust sampled from the parent bodies in column II and collected in a way that would not result in exposure to extreme temperatures.

^eInterplanetary dust sampled from the parent bodies listed in subcolumn Ib.

Scientific Investigations to Reduce Uncertainty:

The task group identified various issues for which scientific research could help to reduce the uncertainty in its assessment of the potential for a living entity to be contained in or on samples returned from planetary satellites and small solar system bodies. (these general suggestions are incorporated into the text of Chapters 2-6) However, one topic is of sufficient importance that it requires emphasis.

Because organisms subjected to sterilizing conditions for a sufficient time period pose no threat to terrestrial ecosystems, it is important to assemble a database on the survival capacity of a wide range of terrestrial organisms under extreme conditions. Despite the existence of a rich literature on the survival of microorganisms exposed to radiation and high temperatures, the studied taxa represent only a small sampling of the microbial diversity known to exist in the biosphere and, in general, have not been taken from extreme environments. Little is known about the radiation and temperature resistance of microorganisms from environments on Earth that have the chemical and physical characteristics likely to be encountered in or on small solar system bodies.

Recommendation: NASA should sponsor research that will lead to a better understanding of the radiation and temperature resistance of microorganisms from environments on Earth that have the chemical and physical characteristics likely to be encountered in or on small solar system bodies. Information on the survival of organisms subjected to long- or short-term ionizing radiation needs to be collected for both metabolically active and dormant stages of diverse groups of microorganisms, including hyperthermophiles, oligotrophic chemoorganotrophs, and chemolithoautotrophs. Likewise, it is important to establish short - and long-term temperature survival curves for similarly broad groups of metabolically active and dormant organisms. In particular, data are required on survival of diverse microorganisms under flash heating (1- to 10-second exposures) to temperatures between 160 C and 400 C.

Summary of: “Mars Sample Handling and Requirements Panel (MSHARP) Final Report,” Michael H. Carr, et. al., NASA, Jet Propulsion Lab, Pasadena, CA, NASA TM-1999-209145 (1999).

Charter: In anticipation of the return of samples from Mars, NASA’s Office of Space Sciences chartered a panel to examine how Mars samples should be handled. The panel was to make recommendations in three areas: 1) sample collection and transport back to Earth; 2) certification of the samples as non-hazardous; and 3) sample receiving, curation, and distribution. This report summarizes the findings of that panel.

Background: The samples should be treated as hazardous until proven otherwise. They are to be sealed within a canister on Mars, and the canister is not to be opened until within a Biosafety Hazard Level 4 (BSL-4) containment facility here on Earth. This facility must also meet or exceed the cleanliness requirements of the Johnson Space Center (JSC) facility for curation of extraterrestrial materials. A containment facility meeting both these requirements does not yet exist. Hazard assessment and life detection experiments are to be done at the containment facility, while geochemical characterization is being performed on a sterilized subset of the samples released to the science community. When and if the samples are proven harmless, they are to be transferred to a curation facility, such as that at JSC.

Summary and Conclusions:

1. The search for evidence of life, particularly past life, is a primary objective of the Mars exploration program. Parallel and intimately connected goals are determination of the planet's climate and of the planet's geologic histories.
2. Many of the outstanding biologic, climatologic, and geologic issues with respect to Mars are unlikely to be resolved until we have a variety of resumed samples.
3. The present martian surface is very hostile to life because of its low temperatures, the lack of liquid water, the high UV flux, the presence of oxidants, and the scarcity of organics.
4. The chances of finding extant life in samples returned from the martian surface are very low, and even if extant life were present, it would be unlikely to have significant ecological impact or other harmful effects on the Earth. The risk is not zero, however.
5. Because we cannot demonstrate that the risk is zero, the returned samples should be assumed to be potentially harmful until proven otherwise. They should be placed in sealed containers on Mars, and the containers should be opened only in a BSL-4 containment facility here on Earth. No samples should leave BSL-4 containment unless sterilized or proven to be harmless.
6. Return of samples to the International Space Station is impractical and is likely to be more risky than returning them to Earth.
7. Sterilizing samples at Mars is not advocated because sterilization would be difficult to accomplish and verify remotely on Mars, and sterilization would destroy much of the biologic and climatologic information in the samples.
8. We endorse the current Athena sample acquisition plan to use a rover to acquire primarily rock cores, with a few additional soil samples. We strongly advocate acquisition of a contingency sample by the lander, although this need not be returned if the rover mission is successful.
9. The sampling strategy should be aimed at acquiring the maximum variety of samples from the sites visited.
10. Contamination of the samples with terrestrial materials is of considerable concern because it could compromise the science results from the samples. Also, any false positives on hazard assessment and life detection tests would confuse interpretation of analytical results from the samples and could significantly delay release of unsterilized samples from BSL-4 containment for distribution to the science community.

11. All components that land on the martian surface must be cleaned to at least Pathfinder levels of cleanliness.
12. All spacecraft components that touch the samples must be sterilized and cleaned to significantly higher standards than Pathfinder.
13. Recognizing that some contamination of the samples could occur, we strongly advocate the use of tracers, witness plates, and assays to help identify adventitious contaminants. We do not, however, advocate deliberately impregnating the drill bits with tracers because of concerns that contamination of the samples by the tracers would be significant and would interfere with sample analysis.
14. The sample canister must be sealed before leaving the martian surface, and the integrity of the seal should be confirmed either before leaving the martian surface or while in orbit at Mars.
15. The sample canister must be transferred to the Earth Return Vehicle (ERV) in such a way that the only martian materials on the ERV are those sealed within the sample canister.
16. Insofar as it is practical during return to Earth, the samples should be maintained at temperatures no higher than 240 K, the maximum temperature they are likely to have experienced on Mars. It is especially desirable that the samples not be allowed to experience temperatures above 270 K.
17. We recommend that introduction of unsterilized material into the Earth's environment be kept to a very low probability, mainly by system design, such as by multiple seals and interleaved filters, rather than through monitoring containment and incorporating various contingency responses into the design. We believe the most likely times of containment failure are at the surface of Mars, when a decision could be made not to return the samples, and during entry and landing at Earth, when monitoring has little value. Limited resources are better used by designing against failure rather than by monitoring and contingency mechanisms.
18. After reaching Earth, the sample canister must be opened in a sample receiving facility (SRF) with the equivalent of BSL-4 containment. The facility must also meet the cleanliness standard used for handling extraterrestrial materials at JSC. To our knowledge, no such facility now exists.
19. We view the SRF as primarily a service facility for the science community, rather than a research facility. The facility will make an early inventory of the samples, do some preliminary hazard assessment and life detection testing, and sterilize a subset of the samples for distribution to the science community for geochemical characterization.
20. Early distribution of a subset of sterilized samples is an essential element in both scientific analysis of the samples and in assessing their potential for harm. The geologic and geochemical characteristics of the samples, such as the presence and nature of any organics, will be important for deciding what hazard and life detection testing needs to be done. Geochemical characterization is most reliably and comprehensively done by the at-large science community. Radiation sterilization is the method of choice because of its minimal effects on the geochemical character of the samples. Allocation of the distributed samples should be by the normal NASA Research Announcement (NRA) Peer Review process.
21. Some hazard assessment and life-detection experiments must be done in the SRF. We think it premature to advise how these might best be done, given that technologies will likely evolve considerably between now and 2008 when the first samples return, but we suspect that hazard assessment will primarily involve tissue-cell culture testing rather than tests on whole organisms.
22. Some of the hazard assessment and life-detection experiments could be done at containment facilities other than the SRF by distributing unsterilized samples to other containment facilities using well established procedures for handling and transporting biohazardous materials.
23. The SRF can be scaled, built, and configured in a variety of ways, depending on such factors as what testing is to be done in the facility, as opposed to testing elsewhere, whether the facility

is for Mars samples only or for extraterrestrial materials in general, and how long the Mars sample return program is to last. We believe that an SRF built from modular, modest-sized, commercially available, biosafety laboratories is appropriate for the early sample returns. Should life be detected and/or the samples prove to be hazardous, then more elaborate alternatives could be built.

24. The SRF should be built, staffed, and operational 1-2 years before receipt of the samples.
25. If and when the samples are found to be non-hazardous, the samples should be transferred to a curation facility such as that at Johnson Space Center (JSC).

Summary of: “Size Limits of Very Small Microorganisms: Proceedings of a Workshop,” Space Studies Board, National Research Council, National Academy Press, Washington, D.C. (1999)

Available online: www.nationalacademies.org/ssb/bib1.html

Background: Following the report of possible microfossils ranging in length from 10 to 200 nm in the martian meteorite ALH84001, NASA’s Office of Space Science requested that the National Research Council’s Space Studies Board organize a workshop to provide a forum for discussions of the theoretical minimum size for microorganisms. The Board formed the Steering Group for the Workshop on Size Limits of Very Small Microorganisms, which convened a workshop on October 22-23, 1998 of leading experts in fields relevant to this question.

The workshop was organized into four panels each addressing a set of distinct but related questions relevant to the size limits of very small organisms. Eighteen invited panelists, representing fields ranging from cell biology and molecular genetics to paleontology and mineralogy, joined with other participants in a wide-ranging exploration of minimal cell size and the challenge of interpreting micro- and nano-scale features of sedimentary rocks found on Earth or elsewhere in the solar system. This NRC report contains the proceedings of the Workshop on the Size Limits of Very Small Microorganisms. It includes position papers presented by the individual panelists, arranged by panel, along with a summary, for each of the four sessions, of extensive roundtable discussions that involved all workshop participants.

Findings:

Panel 1 addressed the following questions:

1. What features of biology characterize microorganisms at or near the nanometer scale?
2. Is there a theoretical size limit below which free-living organisms cannot be viable?
3. If we relax the requirement that cells have the biochemical complexity of modern cells, can we model primordial cells well enough to estimate their likely sizes?

Panel 1 Report: Consensus was reached by Panel 1 participants on the following major points, assuming free-living cells with conventional biochemistry:

1. A minimum of about 250 to 450 essential genes are required for viability.
2. The minimal viable cell diameter is expected to lie in the range of 250 to 300 nm.
3. The number of ribosomes required for adequate genome expression is a significant constraint on minimal cell size.
4. If the requirement for conventional biochemistry and genetics is relaxed, especially with reference to primordial or exobiotic self-replicating systems, the possibility of much smaller cells must be considered.

Panel 2 addressed the following questions:

1. Is there a relationship between minimum cell size and environment?
2. Is there a continuum of size and complexity that links conventional bacteria to viruses?
3. What is the phylogenetic distribution of very small bacteria?

Panel 2 Report: Consistent with the theoretical limits articulated by Panel 1, members of Panel 2 reported that:

1. Bacteria with a diameter of 300 to 500 nm are common in oligotrophic environments, but that smaller cells are not.
2. Nanobacteria reported from human and cow blood fall near the lower size limit suggested by cell biologists; however, the much smaller (ca. 50 nm) bodies found in association with these cells may not, themselves, be viable organisms.
3. Observations on archaea indicate that, in general, they have size limits similar to those for bacteria.

Two problems constrain discussions on minimal cell size in natural environments. Commonly used methods of measuring cell size have inherent uncertainties or possibilities of error. Perhaps more important, most cells found in nature cannot be cultivated. Thus, ignorance about biological diversity at small sizes remains large. These problems notwithstanding, it appears that very small size in modern microorganisms is an adaptation for specific environmental circumstance, including stress and scarcity of resources. Primordial organisms may or may not have been tiny, but the smallest organisms known today reside on relatively late branches of the RNA phylogeny.

Panel 3 addressed the following question:

1. Can we understand the processes of fossilization and non-biological processes sufficiently well to differentiate fossils from artifacts in an extraterrestrial rock sample?

Panel 3 Report: Panel 3 reached a general consensus on the following points:

1. Terrestrial rocks contain an observable and interpretable record of biological evolution, but as we go further back into time, that record becomes attenuated and difficult to interpret in detail. Martian samples may actually be better preserved than terrestrial sediments of comparable age, but lack both modern martian organisms for comparison and a more or less continuous fossil record that connects the present with early planetary history.
2. A better understanding of biological signatures in sedimentary rocks is needed, and it is needed before intelligently collected martian samples are returned to Earth. These signatures certainly include fossil morphologies, but they must also include biomarker molecules, isotopic fractionation, and biological mineralization and trace element concentrations. In all cases, improved understanding of biological pattern formation must proceed in tandem with better knowledge of the generative capacity of physical processes.
3. There is both a need and an opportunity to more effectively integrate laboratory and field observations of fossilization processes with investigations of Earth's early sedimentary record. Multidisciplinary investigations are required in exopaleontological research, and there is a need for new technologies that will enhance our ability to obtain chemical information from individual microstructures.

Panel 4 addressed the following questions:

1. Does our current understanding of the processes that led from chemical to biological evolution place constraints on the size of early organisms?
2. If size is not constrained, are there chemical signatures that might record the transition to living systems?

Panel 4 Report: As yet, there is no consensus view of how life originated. There is, however, broad agreement that the first living systems were far simpler than the simplest free-living organisms known today. The concept that life passed through a stage in which RNA, or a polymer much like it, provided both genetic information and catalysis suggests what such a simple organism might have been like. Organisms characterized by such single-biopolymer chemistry could have been minute, perhaps as small as 50 nm in diameter. This means that the minimum size observable in living cells may not be applicable in setting limits for biological detection on Mars and Europa. The earliest organisms on Earth (or elsewhere) would probably be extremely difficult to recognize as fossils.

Conclusions:

Sometime in the next 10-12 years a small sample of martian rock and soil will be returned to Earth. Among the important questions that will be asked of these samples is; Has Mars ever been a biological

planet? Our ability to address this question is directly related to our understanding of the range of morphological features that can be produced by life and by physical processes, as well as the ranges of organic chemicals, mineral forms, and sedimentary rock features that can be generated by biological and by non-biological processes. The results of the workshop make clear a consensus regarding the size and chemical limits of life on Earth.

But, given reasonable uncertainty about whether such features are particular products of terrestrial evolution or universal features of life, the meter stick by which the biogenicity of martian or other planetary samples is measured will likely be knowledge of the limits on physical processes – knowledge that needs to be developed before samples from Mars arrive in the laboratory.

**Summary of: Current State of Controversy About Traces of Ancient Martian Life in Meteorite ALH84001, Allan H. Treiman (L.P.I.) Feb. 2000.
(Instrumentation used keyed as superscripts)**

McKay et al. Hypothesis – Four arguments together suggest that formation of carbonate globules in ALH84001 was associated with martian life [1]. In the globules:

1. Polycyclic Aromatic Hydrocarbons, PAHs (organic material) are martian and characteristic of degraded organic matter.^{mg2}
2. Mineral assemblages and chemical zoning patterns are characteristic of biologic influence.^{e1,s1,s2,s3}
3. Sub-micron magnetite grains have properties indistinguishable from, and unique to, those formed by some Earth bacteria.^{t1,t2,t3,t4}
4. Surfaces are decorated with bacteria-shaped objects, inferred to be mineralized remains of bacteria.^{s1,s1a}

Precondition: Carbonate globules formed at temperatures consistent with life. Unproven, but probably true [2,3].^{e1,mg1,i1}

1. Organics/PAHs are martian and biogenic?
 - *Martian? Probably.*
 - > Martian origin suggested by intimate mixing with carbonate, decrease in abundance near fusion. [1,4,5]^{mg2,x2,x3}
 - > But some contradictory evidence, issue unresolved [4,6-9]^{mg1,mg2,mg3,mg4,i2}
 - > Nearly all organic carbon in ALH84001 is terrestrial [9-11].^{mg1,mg5,mg6,c1}
 - *Biogenic? Unproven/unprovable?*
 - > Similarity to biogenic PAHs inadequately documented.
 - > Similar to PAHs in CM chondrites and IDPs. [12]
 - > Earth weathering/oxidation reduces all PAHs, of any origin, to core molecules [13].^{mg7}
2. Mineral Assemblages
 - *Not diagnostic of biology [3, 14-16]*
3. Nanophase magnetites
 - *BIOGENIC !? Maybe.*
 - > Carbonate globules all include two layers with abundant submicron grains of magnetite in a porous (?) matrix of magnesite carbonate.^{e1,s1,s1a,t1,t2,t3,t4}
 - > ~1/4 of the magnetites are identical to magnetites from magnetosomes of some magnetotactic bacteria: size, shape, form, structural perfection, lack of chemical substituents [17-19].^{t1,t2,t3,t4}
 - > These properties suffice for recognition of magnetites as biogenic, from bacterial magnetosomes [20,21].
 - *BUT...*
 - > Does not explain other 3/4 of submicron magnetite grains.
 - > Does not explain why magnetotactic magnetites are there.
 - Why would magnetotactic bacteria live in rock?
 - If magnetites were transported into rock, how could magnetite-rich layers in globules be so sharp and be so similar through the rock?
 - Abiotic experiments reported to produce magnetites with these “biogenic” properties [16].^{e1,x1,s1b,s2,t1,t3?,t4,c2}

4. Bacteria-Shaped Objects

- *Visually appealing, scientifically weak*
- *Some are inorganic*
 - > Whisker-shaped magnetites, epitaxially aligned magnetites [22,23] ^{s1,s1a,t1,t2,t3}
 - > Lamellar protrusions on mineral surfaces [24,25] ^{s1,s1a}
- *Some may be terrestrial*
 - > Artifacts of sample preparation? [24] ^{s1,s1a}
 - > Terrestrial objects unknown origin? [26] ^{s1}
 - > Earth organisms? [27,28] ^{s1a,s2,sf1,i1,b1-b5}
- *Some are too small*
 - > Objects of diameter <100 nm suspect as bacteria.
 - > These objects suggested to be bacterial appendages or desiccated bacteria [29].
- *Limited data.*
 - > Few images.
 - > No internal structure.
 - > No chemical compositions.
 - > No sense of community structure.
 - > No sense of ecology.

Summary:

1. *No argument has been fully validated.*
2. *Arguments A, B, D weaker than in 1996.*
3. *Argument C (nanophase magnetites) stronger, but still problematic. A plausible abiotic hypothesis is available.*
4. *Having all four arguments be true together seems less likely than any single one be true.*
5. *The nature of scientific evidence.*
 - *Lack of proof is not disproof.*
 - *Lack of disproof is not proof.*

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Instrumentation Applied to Study of ALH84001 (not all in papers cited above):

S. Scanning Electron Microscopy

- s1. Secondary electron imagery – SEM (SEI)
 - s1a. SEI with a field emission electron gun – FEG-SEM
 - S1b. Environmental SEM
- s2. Backscattered electron imagery – BSE
- s3. Chemical analysis by energy dispersive X-ray spectrometry – EDX

E. Electron Microprobe

- e1. Electron microprobe chemical analyses, X-ray dispersive spectrometry – EMP-WDS
- e2. Element abundance mapping

T. Transmission Electron Microscopy

- t1. Brightfield and/or darkfield imagery – TEM
- t2. High-resolution (lattice-scale) TEM – HRTEM
- t3. Selected area electron diffraction – SAED
- t4. Chemical analysis by energy dispersive X-ray spectrometry – AEM

- t5. Chemical structure/elemental valence by electron energy loss near-edge spectrometry – EELNES

X. X-ray Methods

- x1. Powder X-ray Diffraction – XRD
- x2. Chemical structure / elemental valence by X-ray absorption near-edge spectrometry – XANES

I. Ion Beam Methods

- i1. Elemental/isotopic analysis by Secondary Ion Mass Spectrometry – SIMS
- i2. Elemental/isotopic analysis by Time-of-flight SIMS – TOFSIMS
- i3. Elemental/isotopic mapping by SIMS/TOFSIMS

M. Mass Spectrometric Methods

- mt1. Thermal ionization mass spectrometry – TIMS
- mt2. Negative ion TIMS – NTIMS
- mg1. Gas source mass spectrometry
- mg2. Laser desorption, laser ionization – $\mu\text{L}^2\text{MS}$
- mg3. Laser desorption – LDMS
- mg4. Time of Flight LDMS – TOF-LDMS
- mg5. High-performance liquid chromatography / gas chromatography – HPLC/GCMS
- mg6. Accelerator mass spectrometry – AMS
- mg7. Pyrolysis – gas chromatography

O. Optical Methods

- o1. Petrographic microscopy
- o2. Visible/NIR absorption spectroscopy
- o3. Mid-infrared and thermal infrared absorption/emission spectroscopy
- o4. Raman spectroscopy
 - o4a. Mineralogic mapping

o5. Cathodoluminescence spectroscopy

SF. Scanning Force Microscopies

sf1. Atomic Force Microscopy - AFM

MM. Magnetic Methods

mm1. Thermal demagnetization

mm2. Alternating field demagnetization

mm3. Magnetic susceptibility

mm4. Micro scanning SQUID imagery

C. Chemical Methods

c1. High-performance liquid chromatography – HPLC

c2. Hydrothermal experiments

c2a. Cold-seal

c2b. Flow-through

c3. Inductively coupled plasma atomic emission spectroscopy for elemental composition – ICP-AES

N. Nuclear Methods

n1. Instrumental neutron activation analysis – INAA

n2. Radiochemical neutron activation analysis – RNAA

n3. Mössbauer spectroscopy

n4. Nuclear track analysis

B. Biological Methods

b1. Culturing on sterile media

b2. 16s RNA analysis

b3. DNA analysis

b4. unspecified “biochemical methods”

b5. ? Polymerase chain reaction amplification of nucleic acids – PCR ?

END (My apologies for missing your favorite method).

Additional Relevant Reports (in press or in preparation—November 2001)

a. The Quarantine and Certification of Martian Samples, 2001. Committee on Planetary and Lunar Exploration (COMPLEX), Space Studies Board, National Research Council. National Academy Press, Washington, D.C. 132pp.

Unedited Pre-Publication available online: www.nas.edu (Click on Publications -- Reports)
Executive Summary pp 1-12

b. Mars Sample Handling Protocol Workshop Series (for updated information on individual reports, contact Sara Acevedo, NASA Ames Research Center: sacevedo@mail.arc.nasa.gov)

Mars Sample Handling Protocol Workshop Series. Interim Report of the Workshop Series: Workshop 1 Proceedings and Final Report. Race, M.S. and J.D. Rummel (eds.), 2000. NASA/CP-2000-209624, Washington, DC (Oct. 2000)

Mars Sample Handling Protocol Workshop Series. Interim Report of the Workshop Series: Workshop 2 Proceedings and Final Report , Race, M. S., G.T.A. Kovacs, J.D. Rummel, and S. E. Acevedo (eds.), NASA/CP 2001-210923, Washington, D.C. (2001)

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A Draft Test Protocol for Detecting Possible Biohazards in Martian Samples Returned to Earth (Draft Comprehensive Protocol) M.S.Race, D.L. DeVincenzi, J.D. Rummel, & S. E. Acevedo (eds.), NASA/CP-2002- XXXX, in press Spring 2002), Washington DC

c. When Ecologies Collide? Planetary Protection Issues in the Human Exploration of Mars. Workshop Report. (in preparation, Summer 2001). M.E. Criswell, C.P. McKay, and M. Duke (co-chairs and editors).

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