Protocol

Interim Report of the Workshop Series
Workshop 3 Proceedings and Final Report
San Diego, California
March 19-21, 2001

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NASA/CP—2001–211388

Mars Sample Handling Protocol Workshop Series

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December 2001

Acknowledgements

The editors wish to acknowledge the contributions of the Workshop Planning Committee members (Donald DeVincenzi, Glenn MacPherson, Pericles Stabekis, Jack Schad, and Michel Viso), in assembling the diverse group of scientific experts required for the success of Workshop 3. In addition, the presenters of the 'background tutorials' are thanked for providing a firm foundation on which to base the discussions.

Available from:

NASA Center for AeroSpace Information
7121 Standard Drive
Hanover, MD 21076-1320
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703-605-6000

PREFACE

Numerous NASA reports and studies have identified Planetary Protection (PP) as an important part of a Mars Sample Return mission, both for preventing forward- and back-contamination and for ensuring maximal return of scientific information. A key element of PP for sample return missions is the development of guidelines for returned sample containment, 'biomarker' analysis, and biohazard testing. Reports from two previous major studies *[DeVincenzi et al., 1999, and Carr et al., 1999]* have provided preliminary recommendations on specific aspects of handling returned Mars samples including biocontainment, life detection, biohazard testing, sample collection and transportation, certification, and sample receiving, curation, and distribution.

To further refine the requirements for sample hazard testing and the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened an additional Workshop Series beginning in March 2000. The overall goal of the Series is to develop a comprehensive protocol to assess the returned materials for any biological hazard(s) and to safeguard the purity of the samples from possible terrestrial contamination. It is anticipated that the findings of this Workshop Series will: 1) assist NASA's Planetary Protection Officer and senior administrators in preparing for Mars sample return facilities, technology, and operations; 2) serve as a briefing document for advisory groups, regulatory agencies, and other entities that will ultimately establish and review sample return handling policies, requirements, and implementation, and 3) provide recommendations in a form suitable as input for possible future announcements of opportunity soliciting proposals for Mars sample handling.

This document is the report of the third Workshop in this Series. Information herein will ultimately be integrated into a final document from the entire Workshop Series along with information and recommendations from other workshops in the Series. This report builds on the deliberations and findings of the three earlier workshops in the Series *[Workshop 1, Race and Rummel, 2000; Workshop 2, Race et al., 2001; Workshop 2a, Bruch et al., 2001]* which are available from the National Technical Information Service as indicated on the previous page.

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EXECUTIVE SUMMARY

In preparation for missions to Mars that will involve the return of samples to Earth, it will be necessary to prepare for receiving, handling, testing, distribution and archiving of martian materials here on Earth. Previous groups and committees have studied selected aspects of sample return activities, but specific detailed protocols for handling and testing must still be developed. To further refine the requirements for sample hazard testing and to develop the criteria for subsequent release of sample materials from quarantine, the NASA Planetary Protection Officer convened a series of workshops in 2000-2001. The overall objective of the Series is to develop a draft protocol to assess returned martian sample materials for biological hazards, and to safeguard the purity of the samples from possible terrestrial contamination. Although the development of a detailed, comprehensive sample handling protocol is still a long way off, a consensus is emerging from discussions in this Workshop Series about specific requirements of the protocol and important issues to be addressed.

This report provides a record of the proceedings and recommendations of Workshop 3 of the Series, which was held in San Diego, California, March 19-21, 2001. Materials such as the Workshop agenda and participant lists as well as complete citations of all references and a glossary of terms and acronyms appear in the Appendices. Workshop 3 builds on the deliberations and findings of the earlier workshops in the Series, which have been reported separately.[1]

During Workshop 3, five individual sub-groups were formed to discuss the following topics:

- Unifying Properties of Life
- Morphological organization and chemical properties
- Geochemical and geophysical properties
- Chemical Methods
- Cell Biology Methods

In addition, on the final day, there was a focused plenary discussion on the question "What If Life is Detected?" Summaries of the sub-group findings and the plenary discussion follow here; complete reports are included in this document beginning on page 15.[2]

Sub-group 1A: Unifying Properties of Life

Charter: What fundamental, unifying properties of life as we know them may be applied to life detection on martian soil and rock sub-samples, employing the utility of chemical and cellular assays usually exploited to detect or monitor terrestrial biological activity?

Starting Assumptions: Sub-group 1A began with two assumptions for guiding life detection searches: 1) exclusively 'Earth-centric' life detection approaches should be avoided, and

1. Workshop 1: *Race and Rummel, 2000;* Workshop 2: *Race et al. 2001;* Workshop 2a: *Bruch et al. 2001.*
2. The views and findings expressed by these Sub-groups are preliminary in nature and are not intended to represent a consensus of all participants of Workshop 3.

2) selected methods should be able to detect terrestrial life while focusing on truly fundamental features of life such as those associated with structure, complexity, thermodynamics and kinetics. While knowledge of the structural and metabolic intricacies of terrestrial life will be important features to consider, they should not necessarily be searched for directly. Methods should be developed that are not dependent on specific catalytic abilities or carbon chemistry; these methods should be able to detect life on our own planet as a necessary prerequisite to their use in extraterrestrial sites or on extraterrestrial samples.

Universal Properties and 'Measurables' Guiding Life Detection: Sub-group 1A identified two universal properties of life that will be important in guiding non-Earth-centric life detection: catalytic ability and information content (genetics), both of which can be measured and quantified to various levels of acceptability or inferred from other measurements (e.g., life consumes energy, creates waste products, is exothermic, modifies its environment, replicates, evolves and uses thermodynamic disequilibria to build and maintain other thermodynamic disequilibria). Because many of the signals will not be easily discerned if the life is not active (e.g., is hibernating or quiescent), or has gone extinct, it will be important to understand which of life's 'signals' are permanent (e.g., which will survive long term in the environment even in the absence of active metabolism), and which are transient and dependent on actively metabolizing life forms.

General Principles Guiding the Search for Life: The Sub-group identified biosignatures as the priority approach and starting point for seeking indications of the structural and (eventually) chemical complexities characteristic of life. Structural biosignatures must be supported by coincident chemical data and other signs to be conclusive. Any indicators (structural and/or chemical) strongly suggestive of inactive or 'past' life should be treated as potentially active life. Finally, the Sub-group highlighted the need to understand and use carbon-centered methods and approaches, and to investigate how these might be applied to other chemical species. There was general agreement that the probability of life based on a chemical species other than carbon was rare, perhaps even not possible, but the definition of life – and the search – should not exclude such possibilities.

Iterative Approach to Life Detection: The Sub-group endorsed the concept of using iterative scanning as a method to build a convincing data set for life detection, with results obtained by one method or approach being used to specify and direct subsequent approaches. For example, indications of 'oddities' in the sample materials (e.g., density non-conformities or other types of physical or chemical disequilibria) will be a crucial first step suggesting questions about molecular or structural complexities for further investigation.

General Issues and Data Needs to Support Life Detection Efforts: The Sub-group discussed the amounts, types and uses of data required to support life detection efforts. Discussions particularly highlighted the importance of *in situ* data collection as part of sample return efforts. *In situ* data will be important for a number of reasons (e.g., selection of samples for return to Earth; analysis of samples on Mars while duplicate samples are on the return trip to Earth; as samples that are clearly free from Earth's contaminants; to identify 'oddities' that might help plan how the samples would be accessed, stored, and initially examined; etc.). Over time, the accumulation of *in situ* data and measurements from multiple missions will also help in understanding each additional sample in a planetary context.

Other important areas of emphasis include the use of terrestrial laboratories and simulations for ground-truthing and testing of non-Earth-centric methods, in particular using samples from difficult-to-reach terrestrial habitats, or where the signals of life are very subtle. In addition, there is a need to determine statistically relevant sampling methods for use with returned samples, at the Sample Receiving Facility (SRF) particularly for detecting microbial life in rocks and soils.

Sub-group 1B: Morphological Organization and Chemical Properties of Life

Charter: If putative martian biota is quite different from terrestrial life, what fundamental morphological organization and chemical properties should be taken into account to maximize future life detection efforts? Are there emerging methods (e.g., X-ray microscopy) that should be considered?

Canonical Traits of Terrestrial Life: While recognizing there is no consensus definition of life, Sub-group 1B identified canonical traits of terrestrial life that can be used for recognition and classification. In addition to being based on carbon chemistry, requiring liquid water, and having the ability to replicate, adapt and evolve, other characteristics of life include: 1) the presence of membranes that enable cells to interface with the environment, 2) metabolic activity (and 'metabolic unity') to capture and utilize energy and undergo autocatalytic synthesis, 3) self-replication and genetic evolution resulting in the capacity for increasing complexity from molecules, cell, and cell/cell and cell/environments interactions, and 4) an ability to alter environments through oxidation/reduction reactions, assimilation of micro- and macronutrients, and production of metabolites. The question remains whether these characteristics are dependent on carbon-based life and if they would also be shared by extraterrestrial life.

Most Likely Biosignatures If Life is Unlike Terrestrial Life: After analyzing the essential characteristics of life as we know it, the Sub-group speculated on a wide range of possible alternatives (e.g., non-carbon based, 'dry life', low-tech life, multiple biochemistries and genetic codes, alternative energy compounds, limits to life, size constraints, etc.) and compiled the following thoughts on biosignatures of extraterrestrial life that may be different from terrestrial life:

- *Microscopic Morphology*: Even a non-carbon based or a carbon-silicon-based lifeform would have morphology and mechanisms for growth and reproduction.
- *Structural Chemistry*: More work needs to done regarding the possible structural complexity that can be built into silica and silica-carbon polymers.
- *Metabolism and Bioenergetics*: More work needs to be done to assess the range of metabolic and energy-generating mechanisms that can occur in the absence of carbon or that are different from those known to occur in terrestrial organisms.
- *Biosynthetic Mechanisms*: All life must have mechanisms to synthesize structural, metabolic, and replicative macromolecules. In non-carbon-based life, there may be biosynthetic mechanisms and pathways that are catalyzed by inorganic metals and minerals, or are dependent on physical gradients, catalytic mineral surfaces, and various energy sources.
- *Isotopic Signatures*: Extraterrestrial life will not necessarily fractionate elements (e.g., selectively utilize different isotopes), in the same manner as terrestrial life. Distinctive patterns in the fractionation of carbon, nitrogen, and sulfur might be particularly important

in assessing the possible origins of organic compounds and various volatiles. Other potentially useful isotopes identified include forms of oxygen as indicators of environmental temperature, carbon isotope fractionation patterns in single organic molecules, and fractionation patterns in transition metals.

- *Geochemical Signatures*: Important geochemical signatures potentially indicative of life include magnetite, other minerals out of equilibrium with their normal distribution in the environment, Redfield-like ratios[3] of key elements found in terrestrial life (C, H, O, N, P, S), and isotopic fractionation patterns.

Conclusions and Specific Recommendations: Because of carbons' abundance and unique chemistry, its likely that any life in the universe will be carbon-based. However, there is serious need to address, either through models or experiments, alternate biochemistries which support life or life processes yet are different from that observed in terrestrial life. There is also a need to compile a library of biosignatures that are indicative of the presence of life regardless of its chemical structure or mode of growth and replication.

Specific research areas recommended for further study by Sub-group 1B include:

- *Detection Methods:* Develop a better understanding of the essential features of life, the 'lowest' forms of life that might reflect early stages in the evolution of life, structural and catalytic characteristics of 'low tech' or 'quite different' forms of life, and how these can be detected in extraterrestrial samples. Also, develop methods for analyzing individual small entities that resemble cells and for performing isotopic, elemental, and structural analyses on single cells.
- *Viable Cells and Biomass:* Further research and development are needed on detection of cells and a determination of their biomass using imaging methods to quantitatively enumerate cells (or a biochemical proxy). So far the methods for isolating single cells have been applied to liquid samples and there is no method for the removal of single cells attached to solid substrates. There is a need to develop methods to detect within single cells evidence of metabolic activity and specific macromolecules, including an analysis of their chemical structure and isotopic signature(s).
- *Growth Rate Determinations:* More research and development are needed to refine methods for estimating microbial growth rates in environmental samples containing low numbers of cells. The combination of molecular methods with microautoradiography promises to be useful in estimating the growth rates of specific taxonomic groups of microorganisms.
- *Metabolic Activities:* More research and development is needed to refine methods for estimating rates of specific metabolic reactions in microbial communities from environmental samples and to identify the metabolic potential in these communities. Promising methods include the use of *in situ* microelectrodes and microcalorimetry, molecular methods to determine metabolic activities associated with specific taxonomic groups, and methods to identify specific genes being transcribed *in situ* by microbial communities.
- *Enzymatic Activities:* The methods that have been developed in microbial ecology focus on enzymes that indicate specific metabolic activity such as nitrogen cycle reactions and the rate of degradation of macromolecular organic compounds. Research should continue in the new, sensitive methods that utilize soluble fluorogenic compounds as a proxy for macromolecules that can detect low levels of extracellular hydrolases in environmental samples.

3. The 'Redfield Ratio' describes the ratio of carbon to nitrogen to phosphorous (C:N:P) found in marine organisms.

Sub-group 1C: Geochemical and Geophysical Properties of Life

Charter: What geochemical and geophysical properties of the entire returned sample must be taken into account to select representative sub-samples? What are the final criteria for representative sub-sample selection and preparation? Approximately how many representative sub-samples may be tested?

Recommendations by Category: Sub-group 1C considered their report and recommendations as supplementary to the earlier comprehensive report on a similar topic from Workshop 1.[4] The Sub-group systematically considered the different types of materials anticipated in returned samples and made recommendations by category as follows:[5]

Gas: If there is more than one gas sample, each sample must be considered separately, and filtered to some low-end size limit (0.02 μm; TBC).[6] The solid material from the filtering process is to be treated as a separate sample for analysis purposes. Filtered gas samples can be released from containment without further testing after appropriate filtration.[7]

Head-space Gas: Head-space gas samples should be obtained from each sealed sample container, saving the pumped-off gas and back-filling the sample container with an inert gas. Each head-gas sample should be considered separately, filtered to some low-end size limit (0.02 μm; TBC) with solid material from the filtering process and should be treated as a separate sample for analytical purposes. Filtered gas samples can be released without further testing after filtration.

Bulk Fines: The Sub-group felt strongly that the process of representative sub-sample selection should not result in loss of contextual or other information (for example, composite cemented grains should be treated individually and separately to preserve phase relationships between individual minerals). Rock fragments greater than 2 millimeters (TBC) in diameter contained in the bulk fines should be removed by hand and treated as separate samples. A 'riffle splitter'[8] or similar device should be used for acquiring representative sub-samples, although more research and development must occur to refine the method for use with martian materials. The group suggested the use of optical, UV, IR, and XRD/XRF analyses on an as-required basis to validate that sub-samples separated by physical methods are indeed representative of the entire sample.

Rock Fragments: Sub-group 1C felt that the term 'rock fragments' should be used in place of the previously used term 'pebbles.' The dust from the rock fragments should be removed and treated as fines (i.e., dry removal by vacuuming). Rock fragments should be sorted by lithology and size classes using non-invasive tests (optical, bulk composition, inclusions, XRD/XRF, etc.), with a less than 10% (TBC) by mass portion of each sub-sample used for testing.

4. Race and Rummel, 2000, pages 15-19, Sub-groups 2 and 4 summaries.
5. For definitions of the categories of sample materials, see the full report from Sub-group 1C beginning on page 26.
6. TBC = To Be Confirmed.
7. To date no decisions have been made concerning specific criteria for sample release from the SRF. The filter pore size used must be conservative and based on the best estimates at the time of the size of the smallest possible life forms.
8. A 'riffle splitter' is a mechanical separation device that is able to split an unconsolidated soil sample into two equal parts which have the same grain size distribution (and presumably composition) as the parent sample.

Cores of Solid Rock: Prior to sampling core material by non-invasive analyses (optical examination, surface multi-spectral imaging, XRF, X-ray tomography), the surfaces should be vacuumed to remove fine-grained material (which should be treated as a separate sample, possibly representative of the bulk mineralogy of the core itself). Two strategies were suggested for acquiring representative samples of core material. The first is to sample a representative portion of the core (perhaps grind away a portion of the core, top to bottom and save the powdered material). The second is to identify and classify the different lithologies contained in the core, and sample a portion of each lithology. Either way, the group could see no certain way to select a statistically representative sample of a solid rock core short of completely powdering the core and randomly sampling the powder. Several sub-samples may be required (one statistical sample per core collected by removing a small but representative portion of the whole core), and additional samples representative of each of the lithologies found in the core.

Soil cores: Soil cores should be treated in the same way as solid rock cores, to the extent that the core material remains consolidated, except that the core itself is not vacuumed to remove fine-grained material.

Sub-group 2A: Chemical Methods for Life Detection

Charter: What are the ranking priorities for sensitive chemical methods to enable detection of low biomass or dormant putative martian biota? What applications of these particular methods render them applicable and reduce the margin of error? What type of controls will be necessary to definitively distinguish potential putative extraterrestrial life from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

Using the limits of the Viking GC/MS instrument as a starting point, Sub-group 2A defined 'low biomass' as a level of putative martian microorganisms less than 10^7 cells per gram of sample and developed a proposed chemical protocol for returned Mars samples with at least this level of sensitivity. The ultimate goal of the various analytical techniques is to detect a single microorganism cell in a gram of sample, which will require that total organic carbon measurements have a detection limit in the range of 10^{-13} grams of total organic carbon per gram of sample.

Proposed Sequential Chemical Methods Protocol: The Sub-group designed a 'Chemical Methods Protocol' following a course from non-destructive observational techniques to increasingly sophisticated analyses; the overall protocol is outlined in a series of four flow charts in figures 1-4, pages 31-33.

The Sub-group suggested that a soil sample be processed first. If any of the chemical tests prove positive on soil, then the other samples would potentially yield positive responses. If the soil sample analyses yield completely negative results then they provide a good baseline control for other sample analyses.

The investigation of samples should begin (see figure 1, page 31) using state-of-the-art optical microscopy to provide information on the basic mineralogy of the samples (soils, pebbles, and cores). Observation of any type of organized complex structures would be further investigated to compile a comprehensive inventory of biogenic elements (C, H, N, O, P, S) present (see figure 3, page 32), as well as the cell biology methods outlined by Sub-group 2B of this Workshop (see page 35).

If no organized complex structures are initially observed, the sample should be further investigated for sub-micron morphology (see figure 2, page 32) with SEM and TEM, focusing on structures larger than 100 nanometers. Attempts should be made to concentrate the structures, and if successful, they should be investigated for elemental, isotopic, and organic content as outlined in figures 3 and 4, pages 32 and 33).

The biological elemental abundances found in any martian sample should be compared with relevant biological elemental ratios in various terrestrial organisms. The isotopic characterization of any detected biological elements should be investigated using ion microprobe-based techniques. If carbon is detected in any sample, it should be characterized with respect to its inorganic and organic carbon components.

Any organic carbon should be investigated for its various constituents using an Organic Characterization (see figure 4, page 33) consisting of both microscale direct analyses and extraction based procedures. Any specific compounds that are detected should be further characterized with respect to their chirality and isotopic composition. In some cases, it may be necessary to use instrumentation that is outside the containment facility if such analyses can significantly enhance the detection of key organic compounds.[9]

Estimated Sample Amounts and Times: The amount(s) of sample needed to carry out the various basic analyses were estimated, with non-destructive analyses consuming no sample, and destructive methods requiring as little as a few micrograms to more than one gram. If no organic carbon is detected in a sample smaller than 2.5 grams, a decision would be required as to whether scaling up the sample amount into the range of several tens-of-grams would be likely to produce any meaningful results. The amount of time required to conduct the various analyses was estimated to range from weeks to a few months using present day methodologies, it is anticipated that improvements in analytical methodologies will result in less time being required when samples are returned to Earth a decade from now.

Life As We Don't Know It: Sub-group 2A also considered 'life as we don't know it,' and identified various possibilities for non-terrestrial biochemistry; for example, it may consist of non-biogenic elements (e.g., Si, Fe, Al?); contain no organic carbon; have structures smaller than 100 nanometers; and/or consist of organic monomers.

9. No decision has been made on whether a single or multiple facilities might be utilized to carry out the sample handling protocols. It is possible that specialized testing equipment or infrastructure at locations separate from the SRF may be used as part of the sample handling protocol, with the presumption that appropriate containment and transportation methods would be used if and when samples are moved between facilities. The Workshop Assumptions (see Appendix A1, assumption 9), state "Sub-samples of selected materials may be allowed outside containment only if they are sterilized first."

The probability that any of these 'alternate' life forms exist is unknown and difficult to evaluate, yet perhaps must remain under consideration. In particular, if there is a possibility of 'metabolism-based' life present now on Mars, then returned samples might need to be stored under an inert gas atmosphere in order to limit 'growth' based on chemical fixation of the carbon dioxide present in the Earth's atmosphere.

Sub-group 2B: Cell Biology Methods for Life Detection

Charter: What are the ranking priorities for sensitive cell biology methods that will enable detection of low biomass or dormant putative martian biota? What methods should be considered to reduce the margin of error? What controls are warranted to be able to definitively distinguish putative martian life and its morphology from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

Sub-group 2B endorsed the methods previously proposed in Workshops 1 and 2, and emphasized a sequence of testing that proceeds from general to specific, from non-destructive to destructive, and that retains as much of the pristine sample as possible for scientific study.

A Search for Complexity: Beginning with a scenario that assumes that life is/was rare on Mars, the Sub-group stressed a strategic approach to detecting life in samples, initially using methods to scan for areas likely to contain life and then concentrating more specific detection methods on those smaller areas. There is a need to develop new detection technology that might be called a 'search for complexity.' Important chemical/physical methods to include in such search algorithms include: Light and Scanning Electron Microscopy; UV Fluorescence/Raman; Broad Band Fluorescence; IR Spectroscopy/Raman; GC/MS; Laser Desorption MS, MALDI, ESI; 3D Tomography; Flow Cytometry; and NMR Cytometry.

Non-Carbon Based Life: The Sub-group also addressed the possibility of non-carbon based life and methods appropriate to evaluate that possibility. Based on properties of life that would hold true for both carbon-based and non-carbon-based life (e.g., utilization of energy; need for catalysis; presence of polymers able to store information), the group identified areas needing refinement through further research and development. These included techniques specific to life detection in rock and soil samples (e.g., calorimetry/micro-calorimetry), and methods useful for looking for substrates capable of demonstrating chemical change (the later would be an area appropriate to micro-array technology development).

Cell Biology Specific Methods: As a complement to the chemical and physical methods used in the search for complexity, the Sub-group recommended the use of a relatively small number of cell biology techniques including standard methods for culture of terrestrial organisms, enrichment culture experiments of potential Mars organisms, and enzyme amplification methods.

Considerations to Reduce the Margin of Error: The Sub-group recommended repeated sampling of the same sample location with different methods as a way to decrease the likelihood of error.

New technology development in methods of sample registry will be needed to enable such multiple technique queries. An effort should be made to ask questions that are interpretable, especially in comparison with existing databases and in relation to positive and negative controls.

Controls: 'Witness plates'[10] should be employed during construction of both spacecraft and sample container to test for possible contaminants. Simulants and spiked simulants should be used during development of methods for life detection, especially to understand how mineral composition and martian oxidants could interfere with various tests. All methods should be validated with known controls of Earth microbes and the variety of biomarkers being considered for testing.

Cell Biology Equipment: Excluding equipment necessary for chemical/physical tests, the cell biology-specific equipment includes: thermal cycler; microtiter plate reader; and micro-arrays linked to computers.

Time and Sample Constraints: While some tests will yield results in a minimum of 90-120 days, more time will be necessary for sample replication and verification. A reasonable time for completion of preliminary analysis would be 6 months. The minimum amount of sample was estimated to be 2.5 grams, however multiple replicates and re-testing needs could push that to 10% of the estimated sample, or 50 grams.

Need for New Technology: Sub-group 2B identified the following areas in need of research and new technology development:

- Miniaturization of many chemical analyses;
- Sample registry;
- Micro-calorimetry;
- Database development;
- Complexity search logic;
- Effect of martian versus inert atmosphere on proposed technology; and
- Cleaning/clean room technology.

Plenary Discussion: What If Life Is Detected?

During a plenary discussion on the final day of the Workshop, the participants focused on the question: "If life is detected in the sample (other than confirmed terrestrial contamination), what are the next steps?"
Rather than develop specific recommendations at this time, the participants focused on identifying issues that need further discussion in advance of sample return. The issues fall into three broad categories: 1) Science and Testing; 2) Facility and Technological; and 3) Policy and Administrative.

10. Controls for forward contamination; used to monitor the bioload on the spacecraft and its components *[see Carr et al., 1999, Appendix B for a description of the use of witness plates].*

Science and Testing Issues Related to Discovery: The participants advocated that no materials should be released from maximum containment if life is discovered in any sample material. In addition, testing should be stopped until a previously constituted scientific oversight committee is able to review the adequacy of the protocol itself and provisions for containment. The discussion generated a long list of issues for consideration ranging from a review of preparation, scanning and testing methods, to verification of biocontainment materials and sterilization techniques, to reconsideration of conditions for banking, storage, transportation, and curation. In addition, it will be important to understand what culture and environmental conditions are required to grow more of the organisms for study in the lab, and what precautions are needed in the process.

While detection of life would undoubtedly lead to an emphasis on further biological study, it will also be important to review the protocol for recommended modifications in physical, geological, and chemical tests of sample materials, adding or deleting tests as needed.

Facility and Technological Concerns: Questions about the adequacy of the Sample Receiving Facility (SRF) must also be addressed including the possible need to add equipment, change operations, review emergency plans or upgrade the facilities in response to what is found. The advisability of allowing distribution of sample material outside the SRF will need to be reconsidered as well.

Policy and Administrative Concerns: If martian life is detected, both short- and long-term policy issues will also arise. The short-term list of concerns generally relate to procedures regarding access to and distribution of sample materials, as well as review and publication of research findings. In anticipation of the discovery of extraterrestrial life, it will be advisable to develop an organized communication plan well in advance to avoid a frenzied, reactive mode of communications with government officials, the scientific community, the mass media, and the public. Any plan that is developed should avoid a NASA-centric focus by including other government agencies, international partners, and external organizations as appropriate. It will also be advisable to anticipate the kinds of questions the public might ask and disclose information early and often to address their concerns, whether scientific or non-scientific.

In the long term, the discovery of extraterrestrial life, whether *in situ* or within returned sample materials, would also have implications beyond science and the SRF *per se*. A discovery would trigger a review of sample return and protocol plans for both robotic and human missions. Legal questions may arise about ownership of the data or the entity itself, potentially compounded by differences in laws between the U.S. and other countries since international partners are involved. Beyond the implications for science, policy and future missions, the discovery of life would have profound significance in societal, ethical, theological, and other realms. In anticipation of a possible discovery, it will be especially important to educate a multidisciplinary cadre of students and scientists prepared to grapple with the many, complicated issues ahead.

Final Notes

This document is the final report and complete record of Workshop 3, but only an interim report of the Mars Sample Handling Workshop Series. It provides a record of the complete Workshop 3

process: the agenda, lists of participants, background tutorials presented (in the form of the viewgraphs used by speakers), as well as summary reports from the five individual sub-groups and the subsequent focused plenary discussion.[11] The report will serve a background information for participants of future Workshops in the Series and any other interested parties. The information in this report will eventually be integrated with additional findings and recommendations from the entire Series. If any portion of this report is to be cited or referenced, it must be with the understanding that this document is neither authoritative nor indicative of any final decision or plans for future Mars missions.

11. The summary reports presented in this document (including tables and figures) reflect the deliberations of each sub-group. The findings are preliminary and there may be inconsistencies between the sub-groups. The views expressed and any conclusions and recommendations reached by the sub-groups do not represent a consensus of all Workshop participants and may not necessarily be consistent with the final report and recommendations to be issued at the conclusion of the Workshop Series. Moreover, no attempt has been made to reconcile differences between sub-groups, nor to determine at this time whether particular suggestions would be feasible for a Mars sample return mission.

INTRODUCTION

For upcoming Mars sample return missions, NASA is committed to following the recommendations developed by the Space Studies Board (SSB) of the National Research Council (NRC) in its report on sample handling and testing *[SSB 1997]*. In particular, the NRC recommended that a) "samples returned from Mars by spacecraft should be contained and treated as potentially hazardous until proven otherwise, and b) "rigorous physical, chemical, and biological analyses [should] confirm that there is no indication of the presence of any exogenous biological entity."

As a step towards specifying the requirements for sample hazard testing and the criteria for subsequent release of sample materials from quarantine, NASA's Planetary Protection Officer convened a Series of Workshops in 2000–2001. The stated objective for this Workshop Series is:

> *"For returned Mars samples, develop a recommended list of comprehensive tests, and their sequential order, that will be performed to fulfill the NRC recommendations that 'rigorous analyses determine that the materials do not contain any biological hazards.'"*

This report, which provides a record of the proceedings and findings of Workshop 3 of the Mars Sample Handling Workshop Series, builds on the deliberations and findings of earlier workshops in the Series. The final reports of Workshops 1, 2, and 2a in the Series provide complete information on the basic assumptions, deliberations, and recommendations on specific topics addressed by sub-groups at each of those Workshops.[12]

At Workshop 3, convened March 19-21, 2001 in San Diego, California, the main work occurred in sub-group discussions.[13] Workshop participants were divided into sub-groups to address five separate topics and to develop recommendations as appropriate.[14] On Day 1, the Sub-groups' assigned topics were:

- Unifying properties of life
- Morphological organization and chemical properties
- Geochemical and geophysical properties

After summary reports for each sub-group were presented in a plenary session on the second day of the workshop, participants were re-assigned to sub-groups to discuss two additional topics:

- Chemical methods
- Cell biology methods

12. Workshop 1: *Race and Rummel, 2000;* Workshop 2: *Race, et al. 2001;* Workshop 2a: *Bruch, et al. 2001.*
13. As in previous workshops, Workshop 3 participants were divided into sub-groups based on their background and area(s) of expertise, and assigned topics to be discussed. On Day 1, three sub-groups were formed and met approximately 4 hours to discuss their assigned topics. On Day 2, participants were divided into two additional sub-groups that met for 4 hours of in-depth discussion. All sub-groups reported a summary of their deliberations to the entire body of participants in plenary discussion sessions. Finally, on Day 3, there was a special one-hour plenary discussion addressing the topic 'what if life is detected?"
14. The specific charters of each sub-group and their complete sub-group summary reports are present in detail beginning on page 26 of this report.

After presentation of the two additional sub-group reports in plenary session on the final day, there was a focused plenary discussion on the topic of 'What If Life is Discovered.' The complete reports of all the Sub-groups and the final plenary discussion begin on page 15 of this report.

This document is the final report of Workshop 3, but only an interim report of the Workshop Series. It provides a record of the complete Workshop 3 process: the agenda, lists of participants, background tutorials presented (in the form of the viewgraphs used by speakers), summary reports from the five individual sub-groups, and the subsequent focused plenary discussion.[15]

Ultimately, the information contained in this report will be integrated with information and recommendations that emerge from the other Workshops in the Series. A Final Report for the overall Workshop Series will be published at the conclusion of the Series following review by a science advisory group (see Appendix C3, page 59). *If any portion of this report is to be cited or referenced, it must be with the understanding that this document is not indicative of any final decisions or plans for future Mars missions.*

15. The summary reports presented in this document (including tables and figures) reflect the deliberations of each sub-group. Their findings are preliminary and there may be inconsistencies between the sub-groups. The views expressed and any conclusions and recommendations reached by the sub-groups do not represent a consensus of all Workshop participants and may not necessarily be consistent with the final protocol and recommendations to be issued at the conclusion of the Workshop Series. See the Final Notes on page 11 in the Executive Summary for additional comments.

SUB-GROUP SUMMARY REPORTS

Sub-Group 1A: "Unifying Properties of Life"

Charter

What fundamental, unifying properties of life as we know them may be applied to life detection on martian soil and rock sub-samples, employing the utility of chemical and cellular assays usually exploited to detect or monitor terrestrial biological activity?

The members of this Sub-group were:

Kenneth Nealson (Chairperson)
David J.D. Sourdive (Co-Chairperson)
Gregory T.A. Kovacs
David A. Relman
Mitchell L. Sogin
Andrew Steele
Michel Viso
Norman Wainwright
Mohan Wali

The Sub-group's spirited discussion about the unifying properties of life had a number of themes running through it. The themes, as summarized here in a general form, will hopefully serve as catalysts for future more detailed discussion and decisions. After some discussion, the Sub-group came to the set of starting assumptions summarized in Table 1. These included the notion that, while one must use what is known about terrestrial life to guide our thinking, exclusively 'Earth-centric' life detection approaches should be avoided. The reasons for this general approach were many, but the major concern was that if extraterrestrial life is different from that which is known on Earth, a reliance on Earth-centric methods might possibly cause us to miss it. Thus, Sub-group 1A tried to focus on defining the truly fundamental features of life – those features that could be used to identify any life, but which would always give a positive result with terrestrial life. Focusing on the truly fundamental features of life allows one considerable range in terms of development of approaches. However by stipulating that the selected method must be able to detect terrestrial life, it ensures that a valid ground-truth methodology will be employed.

Table 1: Starting Assumptions for Sub-group 1A

- Avoid 'Earth-centric' approaches
  + Assignment not limited to life as we know it on Earth
  + Assignment not limited to autotrophic or lithotrophic life forms
  + Martian life is not necessarily carbon-based
  + We may not easily find experimental conditions to grow martian life
  + Avoid the focus on specific terrestrial molecules (e.g., DNA, RNA, proteins, etc.)
  + Avoid the potential 'disaster' of missing evidence for unfamiliar life forms
- Design a general method that will at least recognize terrestrial life
  + Detection of signs of organized life (Complexity)
  + Identification of energy flow (Thermodynamics and Kinetics)

Thus, a definition of life (and an approach for finding life) is sought that is not limited to the specific features of life as it is known on Earth. While current knowledge of the structural and metabolic intricacies of terrestrial life will be used, the search will not be limited to those intricate details directly. No details of metabolism (e.g., no necessity for given autotrophic or lithotrophic pathways, and not even any necessity for a carbon-based chemistry) will be assumed. Additionally, while attempts to grow martian life are laudable, these should not be used to discount the presence of life. The Sub-group was in consensus that life would likely be catalytic and carbon-based. However, there was also agreement that methods could be developed that would not be dependent on specific catalytic abilities or carbon chemistry, and that these methods would be able to detect such life on our own planet as a necessary prerequisite to their use in extraterrestrial sites, or on extraterrestrial samples.

Table 2, below, outlines some of the preliminary deliberations that were made regarding non-Earth-centric life detection. These focused on two central properties of life that were thought to be general and measurable: catalytic ability and information content. Both of these properties can be measured, can be quantified to various levels of acceptability, and are thought to be universal properties of life.

Table 2: Universal Properties of Life: Identifying the Properties of Life

- Life is catalytic
  + There should be significant deviations from what is predicted by chemical kinetics
- Life is genetic
  + There will be some system for storing and propagating information
  + There will be molecular distributions with significant capacity for complexity
- Life replicates and evolves
  + There will be evidence for replication of structures and complexity
  + There will be evidence (structural and chemical) for evolution of form and function

Sub-group 1A then tried to expand on these universal properties to include other related features of life that could be measured or observed in some way. These measurable parameters, as shown in Table 3, represent characteristics of life that can be measured directly or inferred from other measurements.

Table 3: Universal Properties of Life: What are the 'Measurables'?

- Life consumes energy
- Life creates waste products
- Life is exothermic
- Life modifies its environment
- Life replicates
- Life evolves
- Life uses thermodynamic disequilibria to build and maintain other thermodynamic disequilibria (in open systems or within a 'wall')

As can be easily seen in Table 3, nearly all of the 'Universal Properties of Life' listed deal in one way or another with energy – energy consumption, energy conversion, creation of waste products, etc. Most of these are directly measurable, although some of them, such as replication or evolution, will be inferred rather than directly measured.

One important feature of this approach that may make it more difficult than anticipated is that many of these signals will not be easily discerned if the life is not functioning (is hibernating or quiescent), or has gone extinct. One of the very large challenges in seeking to detect life in the samples will be that of understanding which of life's 'signals' are permanent (e.g., which will survive long term in the environment even in the absence of active metabolic life), and which are transient and dependent on actively metabolizing life.

The Sub-group then considered what general principles might guide the effort when searching for life. Table 4 lists a few of the principles that were discussed. The structural biosignatures are, to a certain degree, the first order biosignatures that are indicative of the structural and (eventually) chemical complexities that characterize life. Thus as a first place to begin the search, the identification of structural signs is a proper approach. However, it was noted that while structural biosignatures are powerful starting points, without supporting chemical data, they are not convincing, and certainly not conclusive. The Sub-group also dealt with the notion that some indicators (structural and chemical) might well be strongly suggestive of inactive or 'past' life. Any such indications should be treated as potentially active life. This guiding principle will allow one to search for life that is in the process of being fossilized, as well as that already fossilized, with no need to specify that they are indicative of only past life.

Finally, the Sub-group discussed the general principle of understanding and using carbon-centered methods and approaches, and how these might be applied to other chemical species. While there was general agreement that the probability of life based on another chemical species than carbon was rare, perhaps even not possible, the possibility could not be eliminated, and it was put forward that the definition of life should not in particular exclude such possibilities. With this in mind, some thought as to how the carbon-based methods could be generalized need to be considered.

Table 4: General Principles Guiding the Search for Life

- Emphasize structural signs of life that can be easily detected as a first order task
- Agree that recognition of life will be done by coincidence of several signs
- Inactive or 'past' life would be treated as potentially active life
- Generalize a carbon-centered methodology to other chemical species

The search for life will have as a general feature, the search for structures, but with the strong endorsement that structures alone will not be sufficient. Several other chemical criteria will be needed to strongly support the finding of life. Indications of 'past' or inactive life will be treated as potential indicators of active life. Finally, carbon centered methodologies, which dominate our

present thinking and approaches, need to be generalized to other chemical species whenever possible.

Sub-group 1A suggests one approach as a general method – that of iterative scanning, with results obtained by one method or approach being used to specify and direct any subsequent approaches (see Table 5 below). By such repeated iteration and reiteration, one can build a data base that can detect terrestrial life (using non-Earth-centric methods), while getting a feel for the amounts and types of iteration that are required for convincing detection of life.

Table 5: Iterative Approach to Life Detection

- Molecular size and complexity, etc.
- Seek 'oddities' and deviations from the 'norm'
- Structures
- Chemical distributions
- Evaluate complexities and identify molecular biases
- Evaluate multiple methods for detection of complexities and chemistry
- Iterate and reiterate with various methods

The concept of using iterative scanning as a method to build a convincing data set for life detection formed a centerpiece of our discussion. Sub-group 1A focused on the kind(s) of question(s) that when answered in the affirmative will suggest other types of measurements that can be taken by returning to the same sample or sample sub-sites for more information. For example, indications of 'oddities' in the sample materials will be a crucial first step. These might be density non-conformities or other types of physical or chemical disequilibria that will suggest further questions about molecular or structural complexities. Evidence for such physical or chemical oddities will likely suggest additional specific questions about the sample material in relation to the definition of life.

Several additional issues were raised during the deliberations of Sub-group 1A. One of these concerned the amounts and types of data that would have to gathered to support life detection efforts, and how these data would be used. In particular, there was discussion of the importance of *in situ* data, and how it could be used to enhance eventual sample return. The importance of *in situ* data lies in several areas:

- the selection of the proper samples for return to Earth
- the analysis of samples at the surface of Mars while duplicate samples are on the return trip to Earth
- the measurement of samples that have clearly not been exposed to any of Earth's contaminants before the measurements are made

In addition, this approach allows the use of terrestrial 'labs' for 'ground-truthing' and testing of methods. When the non-Earth-centric methods are ready, the testing and iterations can be done on samples that are difficult to reach or where the signals of life are very subtle. Using these

approaches, tests for life can be conducted, and affirm the accuracy of the methods using our tried and true Earth-centric approaches on the same samples.

In situ analyses will provide several other advantages beyond those discussed above, for example, to help alert the sample return community to any 'oddities' that are possible to detect via *in situ* measurements. That is, any major chemical disequilibria, or structural complexity identified in advance, could serve as key guides in planning how the samples would be accessed, stored, and initially examined. This prior knowledge could have a major impact in terms of preparation for the samples, and sophistication with which samples are dealt with as physical and scientific entities.

Knowing the properties of the samples at any level (simple oddities, chemical or structural complexity, etc.) will also be of great potential value simply for distinguishing which samples should be returned – e.g., which are the most likely to yield signs of past or present life, or of the abodes that are available to life on Mars.

Finally, with the advent of more missions and measurements, the accumulation of *in situ* data and measurements will help us understand each additional sample in a planetary context (e.g., how representative of the planet, region, or local area, are certain types of samples?).

In situ science data will clearly provide important information for sample return. The emphasis should be on science that would define the environment, perhaps give early indications of what kinds of samples had been gathered, allow an optimum selection of samples for return to Earth, and help determine how representative the returned samples are of the planet as a whole.

Table 6 summarizes other issues that were raised in closing discussions. These included first the importance of making simulations whenever possible. Is it possible to simulate both martian environments, and the contaminants that might be introduced into these environments by spacecraft of different types? Such information might well play an important role in defining the strategies used for searching and sample gathering on the surface of Mars. This would include the spatial extent of the sampling, as well as any drilling that might need to be done.

Table 6: General Issues for Discussion

- Gather as much data as possible *in situ*:
  + Recognition of 'oddities' will not be easy or straight-forward
  + Need to have something to compare (terrestrial study sites will be critical)
  + Need to be able to sort and select samples (life, geology, chemistry, physics)
  + Need to have some sense of how global the properties being measured are (e.g. Can they be extrapolated to the planet as a whole?)
- Make simulations on Earth for practice (when possible).
  + As data are obtained, it may be possible to anticipate upcoming problems and make preparations for them (e.g. contaminants from landers, etc.).
- Need to determine statistically relevant sampling methods for use with returned materials.

Finally, the 'Unifying Properties of Life' Sub-group emphasized the importance of developing a sampling strategy for use at the Sample Receiving Facility (SRF) that would yield a statistically valid sample. When looking for microbial life, there is in general no good sampling strategy available for rocks and soils. Attention to this topic would be a very strong contribution to the total program of sample return.

Sub-Group 1B: "Morphological Organization and Chemical Properties of Life"

Charter

If putative martian biota is quite different from terrestrial life, what fundamental morphological organization and chemical properties should be taken into account to maximize future life detection efforts? Are there emerging methods (e.g., X-ray microscopy) that should be considered?

The members of this Sub-group were:

John Baross (Chairperson)
Jacques Grange (Co-Chairperson)
Jeffrey L. Bada
J. Gregory Ferry
Marilyn Fogel
Joseph B. Lambert
Christian Mustin
Arthur B. Pardee

Introduction

Although there is no consensus definition of life, there are canonical traits of terrestrial life that allow for its recognition and classification. These traits include carbon chemistry, a requirement for liquid water, and the ability to replicate, adapt, and evolve. Other characteristics of life include: 1) the presence of membranes that allow cells to interface with the environment; 2) metabolic activity so as to capture and utilize energy and undergo autocatalytic synthesis; 3) self-replication and genetic evolution resulting in the capacity for increasing complexity from molecules, cell, and cell/cell and cell/environments interactions; and 4) an ability to alter environments through oxidation/reduction reactions, assimilation of micro- and macro-nutrients, and production of metabolites. Would these characteristics of terrestrial life also be characteristics of extraterrestrial life and are they dependent on carbon-based life?

The above characteristics are present in extant terrestrial life that has had four billion years of evolution. Very little is known about the stages that led to the development of the biochemical, bioenergetic and metabolic properties that resulted in the first evolving organism. Extraterrestrial life may not have evolved all of the characteristics of present-day terrestrial life and could be 'frozen' in some intermediate stage of evolution or could have taken some other evolutionary direction. It is highly likely that other carbon-based organisms would have a different genetic code and may incorporate different amino acids into proteins. However, it is also highly probable that other carbon-based life would have biomarkers that retain some of the characteristics of biomarkers found in terrestrial organisms. These would include organic polymers that maintain

structural integrity of the cells, isotopic fractionation patterns, biominerals resulting from oxidation/reduction reactions, and environmental characteristics including disequilibrium in the ratios of elements and abundance of specific volatiles including oxygen, ozone and methane.

While terrestrial organisms utilize 20 amino acids in their proteins, 5 bases in their nucleic acids, and fatty acids for membranes, there are great variations in structural permutations and in the specific kinds of amino acids and nucleotides. The fatty acids associated with membranes are thought to be excellent biomarkers because they show enantiomeric excess, repeating structural sub-units and structural isomer preference and thus are readily separated from abiotically formed fatty acids. Besides being composed of carbon, terrestrial life also has morphological characteristics that might include structures involved in growth, replication and transport of nutrients into the cell.

Life is carbon based: Earth life is composed of two biopolymers, nucleic acids and proteins. All terrestrial life arose from a common ancestor and thus shares a common genetic code and metabolic, biosynthetic and bioenergetic pathways. The overall consensus is that if life exists elsewhere it would also be carbon based. The evidence includes the abundance of the elements C, H, and O in the universe and the apparent ease in which in organic compounds, with which organic compounds such as amino acids, can be abiotically synthesized. There is also some consensus that extraterrestrial carbon-based life, if originated separately from terrestrial life, is unlikely to have the same genetic code or use the same 20 amino acids as terrestrial life. At the present time there is little information about the limitations of building polymers out of Si or Al that have the capacity to carry out functions necessary for a living cell. The possibility of clay life as proposed by Cairns-Smith[16] is not taken very seriously as an intermediate step in the origin of life; however there is considerable evidence that organic compounds can bind to clays and that some catalytic and condensation reactions can take place on clays. It is speculative whether early life forms could be carbon-based but intimately associated with clays or other minerals such as pyrite and carbonates.

Life requires liquid water: Can there be life in which solvents other than water or possibly volatiles substitute for water? Many enzymes are known to function and show great stability in organic solvents. It is highly likely that all carbon-based life will require liquid water. This is based on the essential role for water in transport of nutrients, creating and maintaining the structure of macromolecules, and catalytic reactions. Since there is a history of water on Mars it is likely that martian life would also require liquid water. The question also remains whether there can be 'dry' life.

There exists 'metabolic unity': No matter what kind of life is found it will likely derive energy in the same ways as terrestrial life, that is, from light and from chemical oxidation/reduction reactions. Understanding the geochemical properties of specific martian terrain is necessary to determine the potential for the environment to support life and the specific energy-yielding chemical reactions. Some of the end products of metabolism of inorganic compounds and metals are potential biomarkers. These include specific crystal structures of magnetite and other metal oxides and sulfides. One of the models for the origin of life is that metabolic and energetic

16. Cairns-Smith, A.G., 1982. *Genetic Takeover and the Mineral Origins of Life*, Cambridge University Press, Cambridge, U.K., 477 pages.

pathways preceded proteins and nucleic acids. These 'metabolites' existed in hot reducing environments such as hydrothermal vents with the catalytic and synthesis reactions taking place on pyrite and possibly other minerals. The question is can you have living organisms in which all of the bioenergetic, metabolic and biosynthetic reactions involve non-protein catalysts? There is evidence that some of the most ancient enzymes found in organisms involve catalytic reactions that can be accomplished without protein enzymes. Some examples include carbonic anhydrase, formate dehydrogenase, hydrogenase, nitrogenase, and acetyl CoA synthetase. All of these enzymes involve a metal-sulfur core. These and other similar reactions in the absence of protein-enzymes could be indicators of 'low-tech' life.

A single biopolymer similar to RNA was key to the formation of 'low-tech' life: Inherent in this assumption is that life formed 'one step at a time' and not by some mechanism in which there would be simultaneous formation of multi-polymers all interacting together and interdependent for function and replication. What could this polymer-mix be composed of, how could it be synthesized, and what environmental conditions would be needed for this synthesis? DeDuve maintains that there would have to be some proto-metabolic process to support an RNA world.[17]

Biological energy is primarily linked to phosphate bonds: There are many other mechanisms that are energetic including 'proton-pumps' across membranes, pH and Eh gradients, light (visible, UV, and infrared), radiation from decay of heavy elements, possibly heat, osmotic gradients (salt, for example), etc. The different kinds of energy compounds and the evolution of energy-rich organic compounds are important issues in the search for 'different' kinds of life and 'low-tech' life. Phosphate is also essential for structural macromolecules including nucleic acids, phospholipids and storage compounds such as polyhydroxybutyric acid (PHB).

There is a 'unity of biochemistry' in all Earth life: All extant life on Earth appears to have evolved from a common ancestor and thus there is a universal code, with highly conserved metabolic and biosynthetic pathways and protein structure. Inherent in this assumption is that any life that evolves on another solar system body would also show this 'unity of biochemistry' since there appear to be universal rules of evolution that include lateral gene transfer, homogenization of the genome and thus a 'unity of biochemistry,' and 'survival of the fittest.' In the early stages of evolution of life there would be no future for a different biochemical 'life form' that did not have a mechanisms to evolve rapidly and exchange 'genetic material' with other 'life forms' having the same biochemistry. These mechanisms are necessary so as to build a large enough genome for cells to be able to grow independently of other cells and thus to enable rapid adaptation to new environments as they form. At this point, it is conjecture whether multiple biochemistries could exist on the same planet or in the same ecosystem, or how many different biochemistries are possible in a carbon-based world.

Terrestrial life defines the 'limits of all life': During the past 20 years, life has been found in environments previously thought to be too extreme to support life. However, there are still some uncertainties on the limits of life, particularly for limits of temperature and pressure, resistance to radiation, and heavy metals. Because of these uncertainties, many questions arise related to the limits of life. For example, could there be active life at temperatures greater than 150°C, in greater

17. De Duve, C., 1995. "The beginnings of life on Earth." *American Scientist*, 83:428-437.

than 50% solute concentrations, or at other extremes? What intrinsic and extrinsic factors would be necessary for life to exist outside of the limits as currently defined? Are there terrestrial organisms that can survive large impact events and the transit from one solar body to another? How long in evolutionary time did it take for microorganisms to evolve mechanisms for survival at conditions outside their growth range or to develop the protein and DNA repair mechanisms needed to cope with high and low temperature, high radiation levels, desiccation, and oxygen damage?

All life is surrounded by a membrane and thus has shape and size constraints: Cells can be recognized because they have morphological characteristics and generally individual species have a limited range of sizes and shapes and thus can be recognized as living organisms. Morphological evidence for growth and reproduction is also important. Morphologic evidence for replication such as dividing cells, budding and fruiting bodies are definitive characteristics of living organisms. Can it be inferred that non-terrestrial life will replicate in similar manners as terrestrial life, i.e. binary fission, budding, spore formation, etc.? It will be important to examine returned martian samples for evidence of growth and reproduction such as microcolonies and biofilms.

The first cells were small: There are selective advantages to cells being small that include increased surface to volume ratios for more efficient transport of nutrients and the interconnection between genetic, biosynthetic and bioenergetic processes *in vivo*. The smallest 'free-living' cells are approximately 200 nanometers in diameter. These cells have small genomes and reduced numbers of ribosomes. The current theoretical smallest size for a 'free-living' organism is approximately 100 nanometers in diameter. Can there be smaller cells? It is possible that during the early stages of evolution, individual cells were part of a community of other small cells each having a small genome and limited capacity for protein synthesis. In essence, the community together would behave as a single cell. If this is the case, presumably it is possible to have cell-like entities that may be smaller than 100 nanometers in diameter.

Most Likely Biosignatures if Life is Unlike Terrestrial Life

Based on a consensus of the essential features of life as we know it, the Sub-group compiled the following thoughts on biosignatures of extraterrestrial life that may be different from terrestrial life. This life would include both carbon-based life that is different from terrestrial life and non-carbon-based or carbon-silica-based life:

Microscopic Morphology: It is assumed that even a non-carbon based or a carbon-silicon-based life would have morphology and mechanisms (size, shape, structure, morphological indicators of replication or specialized functions such as attachment and motility structures, septa, etc.) for growth and reproduction.

Structural Chemistry: More work needs to be done regarding the possible structural complexity (polymers associated with the cell wall, membrane, attachment and motility structures, etc.) that can be built into silica and silica-carbon polymers.

Metabolism and Bioenergetics: More work needs to be done to assess the range of metabolic and energy-generating mechanisms that can occur in the absence of carbon or that are different

from those presently known to occur in terrestrial organisms. There are specific enzyme catalyzed reactions, such as the reduction of nitrogen that can occur from inorganic reactions. There are also thermodynamic models indicating that the reactions involved in energy and CO_2 reduction pathways can occur in the absence of protein-enzymes.

Biosynthetic Mechanisms: All life must have mechanisms to synthesize structural, metabolic and replicative macromolecules. Carbon-based life utilizes protein-enzymes and to a limited extant, ribozymes (catalytic RNA). The synthesis of macromolecules involves a sequence of reactions that depends on the availability of the basic organic components such as amino acids for proteins. In non-carbon-based life, there may be biosynthetic mechanisms and pathways that are catalyzed by inorganic metals and minerals, or are dependent on physical gradients (temperature, pH, Eh, magnetism), catalytic mineral surfaces, and various energy sources (UV and other forms of radiation and light).

Isotopic Signatures: The assumption is that all life will fractionate various elements and that the fractionation pattern will be indicative of life. Many different metabolic groups of organisms show distinctive patterns in the fractionation of carbon, nitrogen, and sulfur. This might be particularly important in assessing the possible origins of organic compounds and various volatiles such as methane, carbon dioxide and carbon monoxide, if detected on Mars. However, it cannot be assumed that extraterrestrial life, particularly if biochemically different from terrestrial life, will fractionate elements in the same manner as terrestrial life.

Other isotopes, such as those for oxygen (detected in carbon dioxide and phosphate), can be indicators of environmental temperature. There is promising new technology for measuring carbon isotope fractionation patterns in single organic molecules and fractionation patterns in transition metals. The later may be very important in identifying a biological source for various minerals such as magnetite.

Geochemical Signatures: Important geochemical signatures include the presence of magnetite, other minerals out of equilibrium with their normal distribution in the environment, Redfield-like ratios of key elements found in terrestrial life (C, H, O, N, P, S), and isotopic fractionation patterns.[18] When specific biologically important elements are limited in the environment, there will be higher concentrations associated with cells or colonies of cells. Usually, the limiting element in the environment will limit the extent of growth and productivity of organisms (known as Liebig's Law of the Minimum). Some key elements that are limited in terrestrial environments include iron, molybdenum, (essential for nitrogen cycle reactions), and tungsten for specific enzymes in hyperthermophilic archaea.

Specific Recommendations

Detection Methods: One of the recommendations of this Sub-group is to better understand the essential features of life, the 'lowest' forms of life that might reflect early stages in the evolution of life, structural and catalytic characteristics of 'low tech' or 'quite' different' forms of life, and how these can be detected from extraterrestrial samples and particularly samples from Mars. While the emphasis will be on identifying an entity that is living, it cannot necessarily be assumed that life

18. The 'Redfield Ratio' describes the ratio of carbon to nitrogen to phosphorous (C:N:P) found in marine organisms.

will be enclosed into some structure and thus resemble terrestrial life. Detection of true extraterrestrial life may be difficult if the emphasis is on techniques that measure morphology or rely on molecular and culture techniques developed for terrestrial life. There is clearly a need to develop methods for analyzing individual small entities that resemble cells. These include the methods to remove individual cells from soil and rocks and ability to perform isotopic, elemental and structural analyses on single cells.

The committee also recommends that there be a thorough study of the geological and geochemical characteristics of the environment from which the sample will be obtained. Evidence for liquid water in the past and deposits of specific minerals, such as magnetite, elemental sulfur and sulfides, phosphates (e.g., apatite), carbonates and silicates, can be indicators of past or present life.

Viable Cells and Biomass: The detection of cells and a determination of their biomass can be measured either using methods that quantitatively enumerate cells in a sample using imaging methods or by using a biochemical proxy for the number of cells. Some biochemical methods, such as those that measure specific fatty acids, can be used to approximate the number of cells in a sample and are very useful in detecting low numbers of 'free living' cells or cells attached to solid substrates. In some cases, it is advantageous to dislodge microbes from particles, sediment grains and rocks in order to get quantitative biomass results. Microbes are dislodged from solid material by first grinding the samples with a mortar and pestle and/or using detergents and mild sonication. Other methods are available for isolating a single cell from a sample. These involve the use of micro-manipulators and lasers to direct single viable cells into capillary tubes for subsequent culturing in defined media or for single-cell Polymerase Chain Reaction (PCR) analyses. So far these methods have been applied to liquid samples and there is no method reported for the removal of a single cell that may be attached to a solid substrate. There is a need to develop methods for the detection within single cells for evidence of metabolic activity and of specific macromolecules including an analysis of their chemical structure and isotopic signature.

Growth Rate Determinations: The ultimate test for viability of cells is their ability to grow and divide. Most of the methods are designed for terrestrial organisms using radio-labeled compounds based on the rate of synthesis of DNA, RNA, or proteins or on their ability to grow in 'growth chambers' and in nutrient media. These radioisotope methods are very sensitive and measure growth rates in environmental samples containing low number of cells. Recently, the combination of molecular methods with micro-autoradiography has proven useful in estimating the growth rates of specific taxonomic groups of microorganisms.

Metabolic Activities: Many methods have been developed for use with environmental samples to estimate rates of specific metabolic reactions in microbial communities or to identify their metabolic potential. Most of these methods require manipulation of the environmental sample such as the addition of radio-labeled carbon or energy sources or substrates for specific enzymes. Other methods, including the use of microelectrodes and microcalorimetry can be performed *in situ*. Some of the molecular methods currently available or in the developmental stages allow for the determination of specific metabolic activities associated with specific taxonomic groups of

microorganisms along with the identification of the specific genes being transcribed *in situ* by microbial communities.

Enzymatic Activities: The activity of most enzymes can be measured in environmental samples if the sample contains sufficient levels of active enzymes. The methods that have been developed in microbial ecology focus on enzymes indicative of specific metabolic activity such as nitrogen cycle reactions and the rate of degradation of macromolecular organic compounds such as proteins and carbohydrates that require enzymatic hydrolysis into soluble compounds that can be transported into cells. The new methods that utilize soluble fluorogenic compounds as a proxy for macromolecules are very sensitive and can detect low levels of extracellular hydrolases in environmental samples.

Conclusions

There are few theoretical models or experiments that focus on either carbon-based life that is different from terrestrial life or non-carbon based life. The most accepted view is that any life in the universe will be carbon-based since it is the only kind of life we know. Moreover, not only is carbon one of the most abundant elements in the universe, it has the versatility to easily form bonds with other elements, build complex macromolecules, and form energetically-rich compounds. However, there is a serious need to address, either through models or experiments, alternate carbon biochemistries that are different from terrestrial life but could support life or life processes. This could include a different genetic code, different mechanisms for transcription and translation and the possibility of novel catalytic processes not involving protein enzymes. It is important that there be a consensus about the definition of life and the canonical characteristics of life including life that is different from terrestrial life. There is also a need to compile a list of biosignatures that will detect life regardless of its chemical structure and mode of growth and replication.

Sub-Group 1C: "Geochemical and Geophysical Properties of Life"

Charter

Sub-group 1C was tasked to consider the following three questions:

- What geochemical and geophysical properties of the entire returned sample must be taken into account to select representative sub-samples?
- What are the final criteria for representative sub-sample selection and preparation?
- Approximately how many representative sub-samples may be tested?

The members of this Sub-group were:

Dave Blake (Chairperson)
Jean-Pierre Bibring (Co-Chairperson)
Dave Beaty
Geoffrey Briggs
David Lindstrom
John Nicholaides III
Michael Singer
Alan Treiman

Sub-group 1C recognized that a fairly comprehensive report on a similar topic was presented in the report from Workshop 1 of the Series *[Race and Rummel, 2000, pages 15-19]*, and the results of the present report should be considered as supplementary to that report. The Sub-group agreed that minimal destruction of sample information is desirable, while assuring a representative statistical sampling of the material for life detection and biohazard testing. The Sub-group considered that the following types of samples[19] would be returned:

1. Gas: If there is more than one gas sample, each sample must be considered separately. Each sample should be filtered to some low-end size limit (0.02 μm, to be confirmed (TBC)). The solid material from the filtering process should be treated as a separate sample for analysis purposes.
   - *Properties taken into account to select representative sub-samples?* None.
   - *What are the final criteria for representative sub-sample selection and preparation?* N/A
   - *Approximately how many representative sub-samples may be tested?* None – filtered gas samples can be released without further testing after filtration.

2. Head-space gas: A head-space gas sample should be obtained from each sealed sample container, perhaps by pulling a vacuum on the sample, saving the pumped-off gas and back-filling the sample container with an inert gas. Each head-space gas sample should be considered separately. The gas samples should be filtered to some low-end size limit (0.02 μm, TBC) and the solid material from the filtering process should be treated as a separate sample for analysis purposes.
   - *Properties taken into account to select representative sub-samples?* None.
   - *What are the final criteria for representative sub-sample selection and preparation?* N/A.
   - *Approximately how many representative sub-samples may be tested?* None – filtered gas samples can be released without further testing after filtration.

3. Bulk Fines (soil): The Sub-group felt strongly that the process of representative sub-sample selection should not result in loss of contextural or other information. For example, if there are composite cemented grains in the sample container, these grains should be treated individually and separately so that the phase relationships between individual minerals are preserved. Rock fragments greater than 2 millimeters in diameter (TBC) contained in the bulk fines should be removed by hand and treated as separate samples.

   The use of a 'riffle splitter' (or something technologically superior to it) was recommended for acquiring representative sub-samples of each sample.[20] The use of a riffle splitter is technically defensible, but needs research and development. It is vulnerable to selective sample loss and is difficult to clean. In addition, the mechanical jostling of the riffle splitter may disaggregate composite grains during the splitting process, thus losing that information.

19. Gas: A sample of Mars atmosphere, collected and stored separately from the solid materials.
    Head-space gas: Mars atmosphere contained in the head space above a solid sample.
    Bulk Fines (soil): Solid unconsolidated materials smaller than about 2 millimeters.
    Rock Fragments: Rocky material larger than about 2 millimeters.
    Cores of solid rock: Consolidated rock cores retaining depth information from Mars surface rocky materials.
    Soil cores: Loosely consolidated soil having some vestige of its original stratigraphy retained.
20. A riffle splitter is a mechanical separation device that is able to split an unconsolidated soil sample into two equal parts which have the same grain size distribution (and presumably composition) as the parent sample.

- *Properties taken into account to select representative sub-samples?* Only physical separation is proposed.
- *What are the final criteria for representative sub-sample selection and preparation?* For bulk fines (soil), it is necessary to determine the extent to which individual sub-samples represent the original sample. The Sub-group suggested the use of optical, UV, IR, and XRD/XRF analyses on an as-required basis to perform sub-sample validation. Perhaps ten sub-samples of each sample could be analyzed/compared in the beginning, until the preliminary characterization team is satisfied that the technique used for physical splitting is statistically valid. These tests are required to provide an empirical basis for the assumption that sub-samples separated by physical methods are indeed representative of the entire sample, physically, chemically and in all other ways.
- *Approximately how many representative sub-samples may be tested?* One for each sample, after validation of the physical sample splitting technique, described above.

4. Rock Fragments: The Sub-group felt that the term 'rock fragments' should be used in place of the term 'pebbles' (used previously) since the latter are defined in the soil science literature as 'rounded or partially rounded rock or mineral fragments 2-75 millimeter in diameter.' 'Rock fragments' are unattached pieces of rock, 2 millimeter in diameter or larger (to be confirmed, TBC), that are strongly cemented or more resistant to rupture. Rock fragments can be spherical, cubic, equi-axial or even flat.

   Because of the way samples are stored during the return trip from Mars, there will likely be coatings of dust unrelated to the rock fragments that are clinging to the outer surfaces of the rock fragments. The dust from the rock fragments should be removed and treated as fines (the Sub-group suggested dry removal by vacuuming).

   The rock fragments should be sorted by lithology (i.e., rock type) and size using non-invasive tests (e.g., optical, bulk composition, inclusions, etc. XRD/XRF etc.).

   - *Properties taken into account to select representative sub-samples?* Size and lithology.
   - *What are the final criteria for representative sub-sample selection and preparation?* A matrix should be made of lithology versus size. For example, if there are 4 different lithologic types (i.e., basalt, sandstone, carbonate, iron oxide), divided into 4 size classes, there will be a matrix of 16 different sub-samples. A less than10% (TBC) by mass portion of each sub-sample should be used for testing.
   - *Approximately how many representative sub-samples may be tested?* One for each size/lithology category (in the above case, 16 separate sub-samples would be tested, comprising in total no more than 10% of each sub-sample).

5. Cores of solid rock: Prior to sampling core material, the core surfaces should be vacuumed to remove fine-grained material. The fine-grained material removed from each core should be treated as a separate sample, possibly representative of the bulk mineralogy of the core itself (depending on how the core is collected on Mars and stored on the return trip to Earth).

   Two strategies were suggested for acquiring representative samples of core material. The first is to sample a representative portion of the core (perhaps grind away a portion of the core, top to bottom and save the powdered material). The second is to identify and classify the different lithologies contained in the core, and sample a portion of each lithology. Either way, it will be difficult to ensure that a truly representative sample is obtained for testing. The Sub-

group could see no certain way to select a statistically representative sample of a solid rock core short of completely powdering the core and randomly sampling the powder.

- *Properties taken into account to select representative sub-samples?* Results of non-invasive analyses: Optical examination, surface multi-spectral imaging, XRF, X-ray tomography.
- *What are the final criteria for representative sub-sample selection and preparation?* Use all available data from (1) above to ensure that a representative sample is obtained.
- *Approximately how many representative sub-samples may be tested?* Several sub-samples – one statistical sample per core (collected by removing a small but representative portion of the whole core), and additional samples representative of each of the lithologies found in the core.

6. Soil cores: Soil cores should be treated in the same way as solid rock cores, to the extent that the core material remains consolidated. The description below is identical to that in (5) above, except that the core itself is not vacuumed to remove fine-grained material. Again, two strategies were suggested for acquiring representative samples of core materials: 1) To sample a representative portion of the core, and 2) To identify and classify the different lithologies contained in the core, and sample a portion of each lithology. The same reservations were expressed as described above for rock cores apply for soil cores: it will be difficult to ensure that a truly statistically representative sample of a soil core is obtained for testing.
   - *Properties taken into account to select representative sub-samples?* Results of non-invasive analyses: Optical examination, surface multi-spectral imaging, XRF, X-ray tomography.
   - *What are the final criteria for representative sub-sample selection and preparation?* Use all available data from (1) above to ensure that a representative sample is obtained.
   - *Approximately how many representative sub-samples may be tested?* Several sub-samples – one statistical sample per core (collected by removing a small but representative portion of the whole core), and additional samples representative of each of the lithologies found in the core.

Sub-Group 2A: "Chemical Methods"

Charter

What are the ranking priorities for sensitive chemical methods to enable detection of low biomass or dormant putative martian biota? What applications of these particular methods render their applicability and reduce the margin of error? What type of controls will be necessary to definitively distinguish potential putative extraterrestrial life from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

The members of this Sub-group were:

Jeffrey L. Bada (Chairperson)
Christian Mustin (Co-Chairperson)
Carl Allen

John Baross
David Beaty
Jean-Pierre Bibring
Geoffrey Briggs
Jacques Grange
Joseph B. Lambert
J. Gregory Ferry
Marilyn Fogel
John J. Nicholaides III
Arthur B. Pardee
Mitchell L. Sogin

Background

Sub-group 2A began by adopting a definition of 'low biomass' based on the limits of the Viking GC/MS instrument for the detectability of bacterial cells in martian soils.[21] 'Low biomass' was defined as a level of putative martian microorganisms of less than 10^7 cells per gram of sample. Therefore, the chemical analytical methods to be applied to samples returned from Mars should have at least this level of sensitivity. The goal of the various analytical techniques should be to push the sensitivity in order to have the ability to ultimately detect a single (!) microorganism cell in a gram of sample. This requires that total organic carbon measurements have a detection limit in the range of 10^{-13} grams of organic carbon per gram of sample. Because they constitute the bulk of the organic carbon in a bacterial cell, amino acid detection limits would need to be at roughly the same level. Most other specific organic components would need to have lower detection limits, in some cases by several orders of magnitude.

Proposed Chemical Methods Protocol

A sequential 'Chemical Methods Protocol' was designed by Sub-group 2A and is described in figures 1 through 4 on the following pages, which follow a course leading to increasingly sophisticated analyses. It was assumed that samples returned from Mars will consist of soil, pebbles, and cores, the Sub-group suggested that a soil sample be processed first as a baseline control sample. If any of the chemical tests prove positive with respect to the type of response expected from putative organisms, then the other samples would likely also have the potential for a positive response. If the soil sample analyses yield completely negative results, then they provide a good baseline control for the analyses of the other types of samples.

A sample should first be investigated using state-of-the-art optical microscopy (see figure 1), which at the very least, would provide information about basic mineralogy of the sample. The observation of any type of organized complex structures would require that the sample be immediately further investigated to inventory the biological elements present and to provide detailed isotopic and organic characterization of the sample (as per the 'Biological Element,

21. It was originally estimated that at least 10^5 microorganisms would need to have been present in 250 mg of martian soil in order to have been detected by the Viking GC/MS (Anderson et al.,1972. *Icarus* 16:111-138). Recent experiments designed to mimic the pyrolysis method used by the Viking GC/MS have found that bacterial cells at a level ~$30x10^6$ cells per gram of soil would likely have been missed by the Viking GC/MS (Glavin et al., 2001. *Earth Planet. Sci. Letts.* 185:1-5). Because a single prokaryotic cell has a dry weight of $2.3x10^{-13}$ g (Neidhardt et al., 1990. *Physiology of the Bacterial Cell*, Sinauer Associates, Inc., Sunderland, MA, 506 pp), this means that several parts per million of organic carbon derived from bacterial cells could have been missed by the Viking GC/MS. Interestingly, this is several orders of magnitude higher than the level of one part per billion that is generally quoted for the amount of organic carbon detectable by the Viking GC/MS instrument in the martian soils.

Isotope, and Organic Characterization' described in figure 3, next page). In addition, samples should be analyzed using the Cell Biology Methods outlined by Sub-group 2B (see page 35).

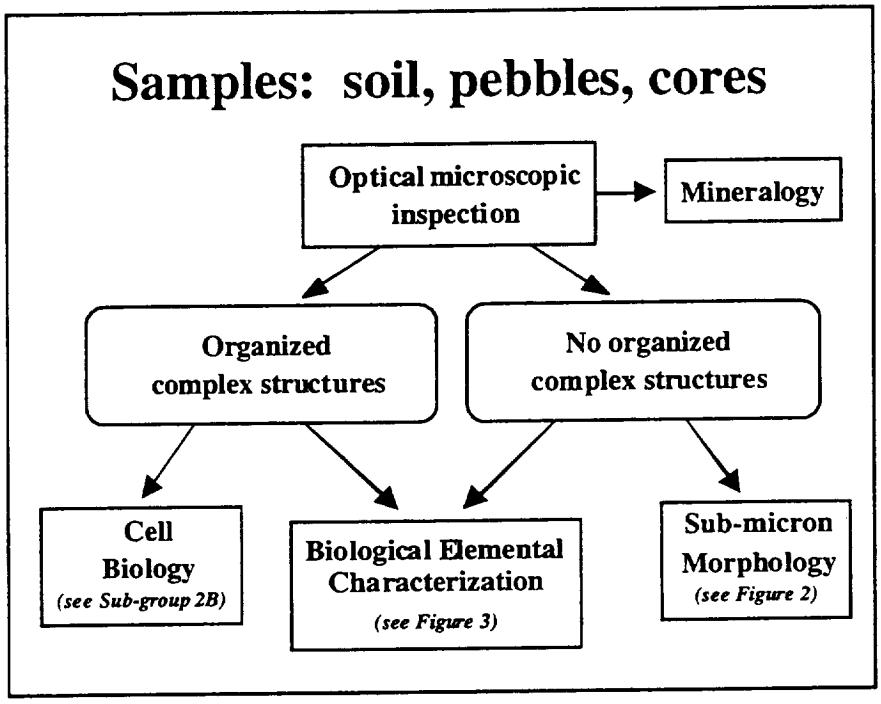

Figure 1. State-of-the-art optical microscopy should be used to search for complex structures.

If no organized complex structures are initially observed, the sample should be further investigated for sub-micron morphology using SEM and TEM (see figure 2, next page). Structures greater than 100 nanometers are of potential interest because this is considered to be the minimal size for organisms with a genome similar to that of terrestrial organisms.[22] Any structures larger than 100 nanometers should be investigated using techniques such as elemental imaging and methodologies used to study microbes present in sub-surface rocks on Earth. Attempts should be made to concentrate the structures, and if successful, they should also be investigated to inventory the biological elements and provide detailed isotopic and organic characterization as outlined in figure 3 on the next page. In addition, the structures should be further analyzed using the Cell Biology Methods outlined by Sub-group 2B (see page 35).

Once the samples or any structures are non-destructively inventoried for their biological elements, destructive analyses should be carried out (see figure 3) in order to obtain precise values for the amounts of the biological elements present, isotopic ratios, and organic content. A useful basis for comparison for the biological elemental abundances found in any martian sample would be the 'average' element ratio of C, H, N, O, P, and S in typical terrestrial microbes. For example,

22. *Size Limits of Very Small Microorganisms*, Space Studies Board, National Research Council, National Academy Press, Washington , D.C., 1999.

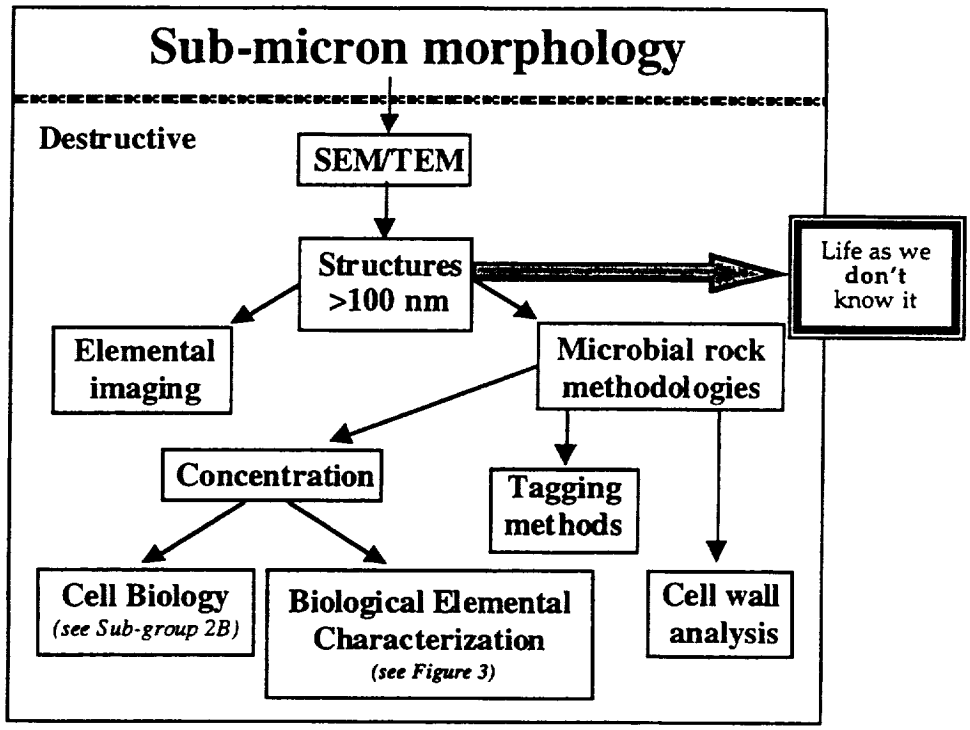

Figure 2. The sample should be further investigated for sub-micron morphology.

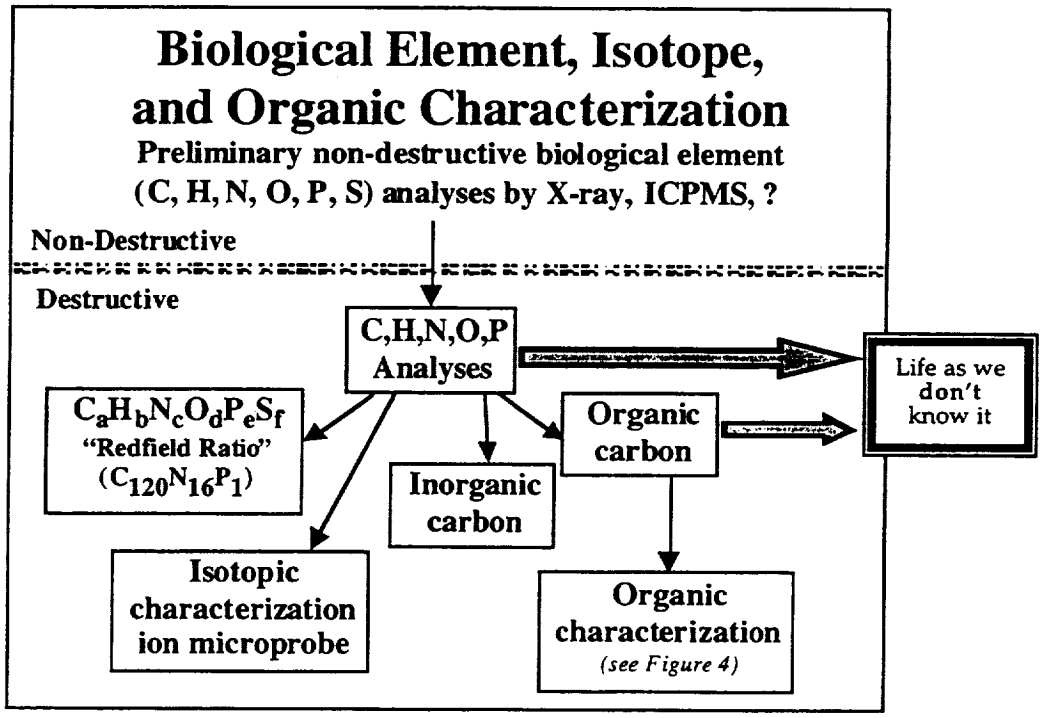

Figure 3. Any structures found should be analyzed for biogenic elements, isotopic ratios, and organics first through non-destructive techniques followed by destructive analyses.

marine organisms have an average ratio of C:N:P of 120:16:1, a parameter known as the 'Redfield Ratio.' Efforts should be made to compile a database of biological elemental ratios in various terrestrial organisms in order to provide a reference for any biological elemental ratios found in martian samples. The isotopic characterization of any detected biological elements should be investigated using ion microprobe based techniques. If carbon is detected in any sample, it should be characterized with respect to its inorganic and organic carbon components. Any organic carbon should be further investigated for its various constituents using the Organic Characterization shown in figure 4 below.

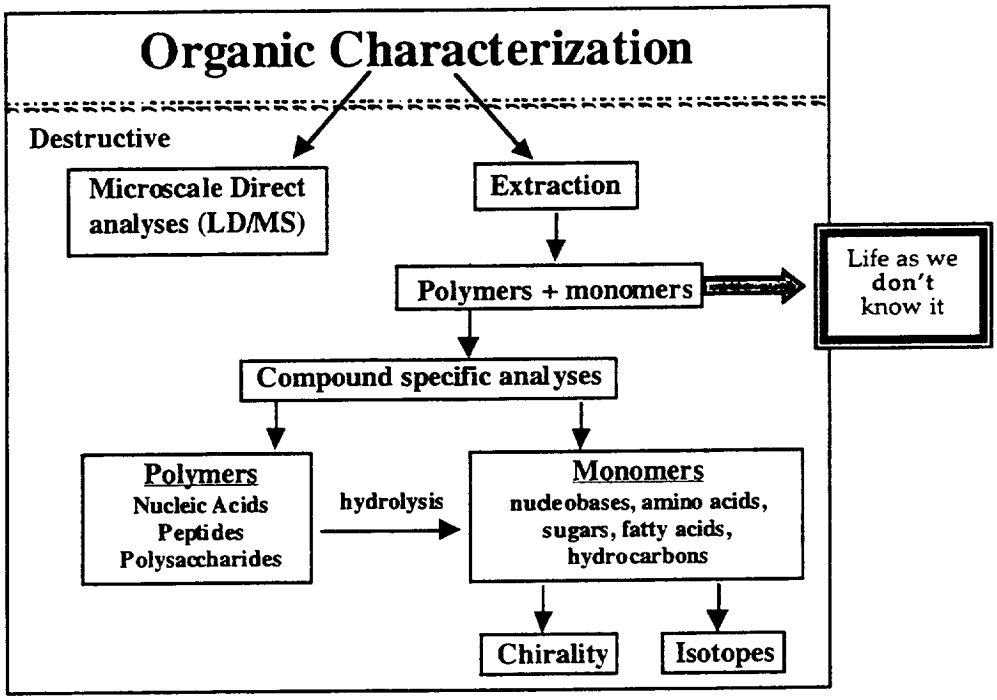

Figure 4. Complete organic analyses is a destructive process.

The Organic Characterization consists of both microscale direct analyses such as LD/MS (considered to be only partly destructive) and extraction based procedures designed to focus on distinct classes of organic compounds. Any extraction procedure should evaluate whether the extract contains a mixture of both polymers and monomers and these should then be dissected into their various constituents. In general, any state-of-the-art analytical method that can be housed within the containment facility should be used in these investigations. Any specific compounds that are detected should be further characterized with respect to their chirality when applicable (for example amino acids, sugars, and some hydrocarbons) and their isotopic composition. In general, the difficulty of analysis increases in going from the top to the bottom of figure 4. In some cases, the detection of specific compounds will be limited by sensitivities of the techniques that are available at the time. It may be necessary to use instrumentation that is outside the containment facility if these analyses can significantly enhance the detectability of

key organic compounds.[23] During the extraction procedures, suitable blanks (for example minerals such as serpentine that has been heated for several hours at 500°C) should be processed simultaneously in order to provide a way to evaluate the signal from terrestrial background contamination.

Estimated Sample Amounts

The amounts of sample needed to carry out some of the basic analyses were estimated assuming that a sample weighing less than 5 grams is split into two halves and replicate analyses are performed. Non-destructive analysis of materials ranging from grains to rocks/cores can be done directly with a number of techniques including optical microscopy, *in situ* microchemical analyses, and microscale direct organic analyses. Destructive analyses of soils and rocks/cores will use varying amounts of materials, estimated as follows:

Inorganic Carbon (0.1- 1%)	few micrograms
Organic Carbon (10^6 cells/g)	1 mg = 10^{-12} moles CO_2
Organic Characterization	> 1 g?
Isotopic Analyses (C,N,O,S,D)	> 1 g?

Specific organic compound analyses as well as isotopic measurements of organic components should only be carried out after the total organic carbon measurements have been completed to ascertain whether sufficient amounts of any particular organic compound might be detectable given the respective sensitivities of the analytical method. If no organic carbon is detected in a sample weighing less than 2.5 grams, a decision should be made about whether scaling up the sample amount into the several tens-of-grams range is likely to produce any meaningful results.

Estimated Time

The time required to conduct some of the basic analyses were estimated using present day methodologies. Presumably, improvements in methodologies in the next decade will translate into shorter amounts of time required by the time martian samples are actually returned to Earth. Biological elemental analyses can presently be carried out in a few weeks using a variety of methodologies. Specific organic compound analyses can, in most cases, be carried out in 1-3 weeks. It is estimated that the various chemical analyses discussed here could be completed in a few months after the returned samples are retrieved on Earth. In general, meaningful baseline data on organic and inorganic carbon amounts could be obtained within a week or so after the samples are delivered to the containment facility. If organic carbon is detected in any sample, specific compound analyses could be completed within a couple of weeks.

23. No decision has been made on whether a single or multiple facilities might be utilized to carry out the sample handling protocols. It is possible that specialized testing equipment or infrastructure at locations separate from the SRF may be used as part of the sample handling protocol, with the presumption that appropriate containment and transportation methods would be used if and when samples are moved between facilities. The Workshop Assumptions (see Appendix A1, assumption 9), state "Sub-samples of selected materials may be allowed outside containment only if they are sterilized first."

Life As We *Don't* Know It

Sub-group 2A also considered the possibilities of dealing with putative martian life which may be vastly different than terrestrial life – 'life as we don't know it.' The possibilities range from putative martian life based on non-biological elements to life based on simple organic monomers. Specifically, the Sub-group identified the following possibilities for 'life as we don't know it':

- Consists of non-biological elements (e.g., Si, Fe, Al?)
- Contains no organic carbon
- Structures smaller than 100 nanometers
- Consists of organic monomers

At this time, the probability that any of these is remotely possible is unknown and difficult to evaluate. Discussions of the possibility of non-carbon based life has had a rich history, especially in the realm of science fiction.[24] So far no one has yet encountered any of the metallic organisms or non-carbon based life forms imagined by science fiction writers, but perhaps they should be considered. Life based on organic monomers has recently been proposed as a model for the 'metabolism-first' scenario for the origin of life.[25] According to this model, a set of self-sustained chemical reactions might be considered 'living' if metabolism is considered to be more important than replication as a fundamental basis of life. If there is serious consideration for the possibility of 'metabolism-based' life present now on Mars, then returned samples might need to be stored under an inert gas atmosphere in order to limit 'growth' based on chemical fixation of the carbon dioxide present in the Earth's atmosphere.

Sub-Group 2B: "Cell Biology Methods"

Charter

What are the ranking priorities for sensitive cell biology methods that will enable detection of low biomass or dormant putative martian biota? What methods should be considered to reduce the margin of error? What controls are warranted to definitively distinguish putative martian life and its morphology from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

The members of this Sub-group were:

Norman Wainwright (Chairperson)
Michel Viso (Co-Chairperson)
David Blake
Gregory T.A. Kovacs
David Lindstrom
Kenneth Nealson
David A. Relman

24. H.G. Wells writing in the *Pall Mall Gazette* in 1894 scolded scientists for thinking of only carbon-based life: "It is narrow materialism that would restrict sentient existence to one series of chemical compounds, and the conception of living creatures with bodies made up of the heavier metallic elements and living in an atmosphere of gaseous sulfur is no means so incredible as it may, at first sight, appear."

25. Wächtershäuser, 2000. *Science* 289, 1307-1308.

Michael J. Singer
David J.D. Sourdive
Andrew Steele
Alan H. Treiman
Mohan Wali

Introduction

Assumptions: There was general agreement that Workshops 1 and 2 on Life Detection methods were sound and summary figures for the previous two Workshop Sub-groups are included for reference (see figures 5 and 6, next page). Many of the methods and techniques stress the need to proceed from general to specific, and non-destructive (or less destructive) to more destructive. A minimum amount of the sample should be used for life detection and biohazard tests so as to retain as much of the pristine sample as possible for scientific study.

A Search for Complexity: A large part of the deliberations stressed a more strategic approach to the detection of life. In one scenario, if life is (or was) a rare occurrence, then the array of chemical detection methods would most productively be used to screen for areas likely to contain life and then concentrate more specific methods on that smaller area. Scanning or screening methods might begin with techniques that could cover large areas of sample material looking for morphology consistent with life, using light and scanning microscopy techniques as well as methods that scan for fluorescence or absorbance signatures. This would minimize time as well as sample. The group felt there is a need to develop new technology in this area that might be called a 'search for complexity.' Such algorithms would be used to focus more intensive methods and therefore increase chances of success.

The following methods were considered important chemical/physical methods to include in search algorithms:

- Light/Scanning Electron Microscopy
- UV Fluorescence/Raman
- Broad Band Fluorescence
- IR Spectroscopy/Raman
- GC/MS
- Laser Desorption MS, MALDI, ESI
- 3D Tomography
- Flow Cytometry
- NMR Cytometry

Research directed at algorithm development should include scanning large surface areas or large volumes of dispersed sample, such as in dust or pulverized rock samples. Particularly relevant would be sequential analysis of a sample by one or multiple techniques. Such analysis would highlight temporal changes due potentially to life chemistries or make logical connections between data obtained by one technique that could focus another technique on the same location.

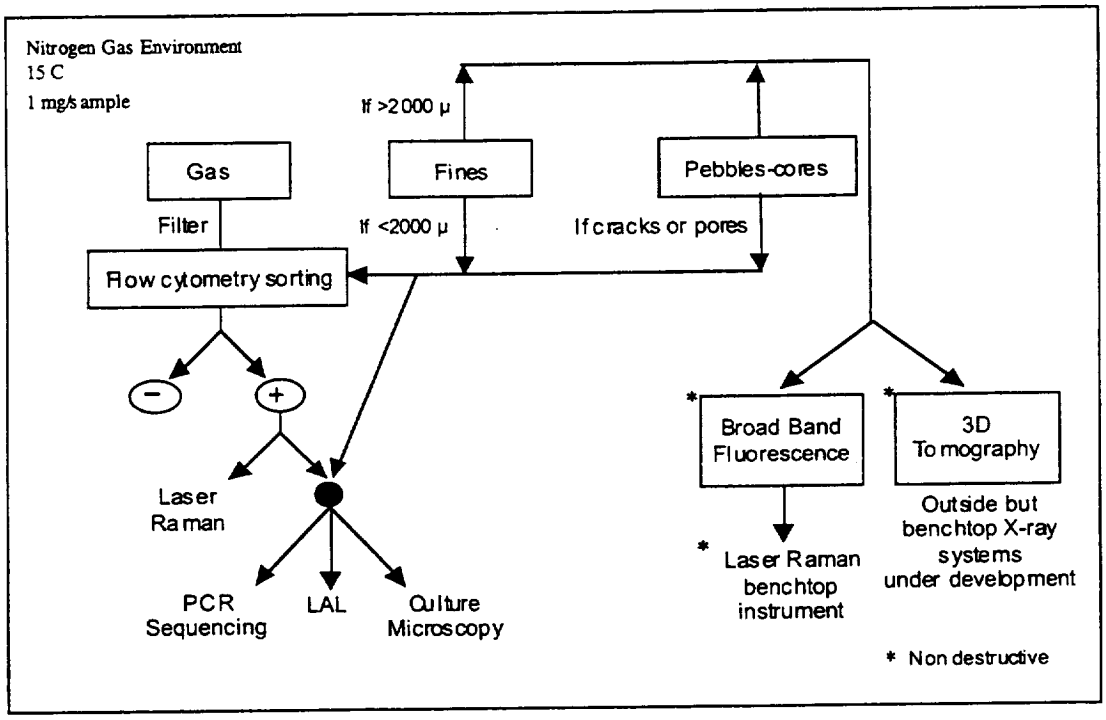

Figure 5: Workshop 1 Life Detection Protocol Flowchart *[Race and Rummel 2000].*

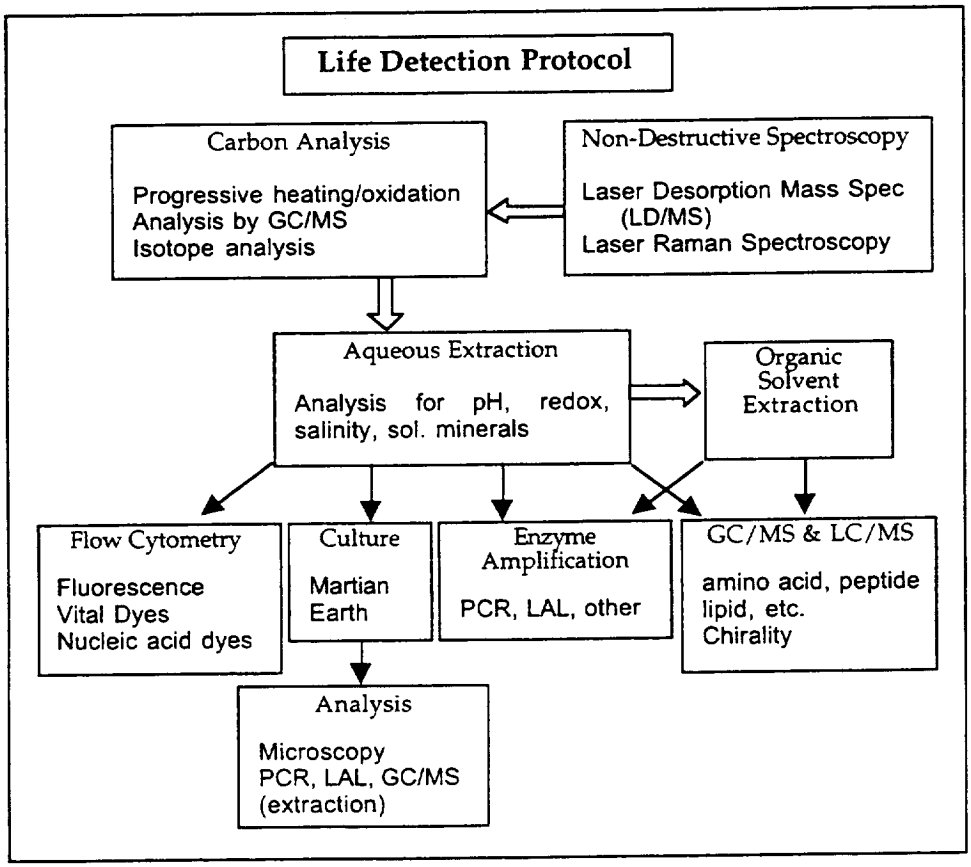

Figure 6: Workshop 2 Life Detection Protocol Flowchart *[Race et al. 2001]*

Non-Carbon Based Life: A second scenario, not addressed in earlier Workshops, was the possibility of non-carbon based life and methods that would be appropriate to evaluate that possibility. Properties of life that would hold true for all carbon based life as well as non-carbon based life include: 1) utilization of energy; 2) the need for catalysis (such as enzymes); and 3) the presence of polymers that could store information

For energy utilization, further development is needed to refine techniques that would be specific to life detection in rock and soil samples including calorimetry/micro-calorimetry. Especially useful would be new methods in calorimetry that could address small areas or scan large areas for specific locations of interest for perturbations of the normal thermal background that would merit further testing.

The presence of catalysts may best be probed by looking for substrates capable of demonstrating chemical change. Micro-array technology, similar to that employed for nucleic acid detection would be appropriate to develop for this application. Hundreds of potential substrates, coupled to colorimetric or fluorometric leaving groups could be exposed to a small quantity of sample. Changes in optical density of fluorescence could be quantified with the appropriate light sources and sensors.

Potential information-bearing polymers may be more difficult to detect. Techniques that separate molecules on size and charge, such as electrophoresis or micro-filtration or sieving, may be applied. Due to the complexities in sample preparation, such techniques would likely be performed on a secondary or corroborative basis.

Cell Biology Specific Methods

Aside from those chemical and physical methods used for searching for complexity compatible for life, there are a relatively small number of Cell Biology techniques to consider:

- Culture
- Enzyme Amplification (e.g., PCR, LAL, ATP, etc.)
- Micro- or Nano-Array
- Nucleic Acids
- Proteins
- Redox pairs (e.g., P, S, N, others)
- Sequential Analysis (i.e., multiple analyses to log changes consistent with life)

Culture of Terrestrial Organisms: Standard planetary protection methods should be applied, including the use of rich medium and standard plating procedures to visualize colony growth. Viable cultures should be analyzed using standard microbiological typing. Extracted nucleic acids should be amplified by PCR using primers known to amplify ribosomal DNA for analysis by sequencing and comparison to sequence databases.

Culture of Potential Mars Organisms: While possible cultivable martian life should be explored, the likelihood and therefore the priority should be low. Enrichment culture experiments should incorporate the chemical analysis of the collection environment. Analysis of 'success' should

include sequential measurements that could detect chemical changes in the culture over time that are compatible with terrestrial biochemistry. This would include gas analysis, calorimetry, and the accumulation of change, such as oxidation or reduction, in medium constituents. These experiments are likely to be long term and 'open-ended.' A positive result for a true non-terrestrial organism in culture would necessitate continued quarantine.

Enzyme Amplification Methods: PCR analysis will be performed on any cultivable organism and efforts may be made to extract nucleic acids from selected samples. While most useful for characterizing Earth contaminants, consideration should be given to methods that explore amplification in the presence of non-standard nucleotides or amplification of RNA-containing organisms.

Limulus Amebocyte Lysate (LAL) is an extremely sensitive method that reacts with cell wall material (lipopolysaccharide and beta glucan). A positive by LAL would necessitate investigation of possible Earth contamination. Other enzymatic methods are in development, including ATP analysis and RNase testing, that would also indicate potential microbial contamination.

Considerations to Reduce the Margin of Error:

- *Multiple Technique Query.* If the same sample location yields a 'positive result' with more than one method, especially if the mechanism of detection is very different, that would decrease the likelihood of error. The Sub-group recommended new technology development for methods of sample registry that enable one to query the exact same area multiple times.

- *Ask questions that are interpretable.* An effort should be made to quantify answers in a way that error bars can be inserted. Also, the Sub-group stressed the significance of asking questions that can be interpreted, especially how results may be compared to existing databases, and the inclusion of positive and negative controls.

Controls:

- 'Witness plates'[26] should be employed during all aspects of spacecraft and biological sample container construction to test for possible contaminants.

- Employ simulants and spiked simulants to develop methods that will be used for life detection. It is important to understand what likely minerals will be in the sample and how its chemical composition could interact either with living organisms or the tests that will detect the signatures of life. Special consideration should be given to oxidants known to occur on the martian surface.

- Methods should be validated with known controls of Earth microbes and the variety of biomarkers being considered for testing. Note should be taken of percent recoveries of viable organisms or biomarkers and consideration given to the amount of sample that may have to be used to overcome such loss.

26. Controls for forward contamination; used to monitor the bioload on the spacecraft and its components *[see Carr et al., 1999, Appendix B for a description of the use of witness plates].*

Cell Biology Equipment: Excluding equipment necessary for chemical/physical tests, the cell biology-specific equipment includes:

- Thermal Cycler
- Microtiter Plate Reader for PCR, LAL, ATP, and RNase methods
- Micro-arrays linked to computers for nucleic acid detection and catalytic analysis

Time and Sample Constraints

Considering the intense scientific and public interest anticipated while preliminary assessment tests are being conducted on returned samples, it will be important to conduct the protocol tests in a prompt manner. While some tests will yield results in a minimum of 90-120 days, more time will be necessary for sample replication and verification. Methods that are investigating culture conditions may take the longest time and may remain ongoing for many months or years. It was felt a reasonable time for preliminary analysis would be 6 months.

Sub-group 1C estimated that the minimum amount of sample for the Cell Biology Methods would be 2.5 grams, however multiple replicates and re-testing needs could push that to 10% of the estimated sample, or 50 grams.

Need for New Technology

The Sub-group identified the following areas in need of continuing and/or new research and technology development:

- Miniaturization of many chemical analyses to minimize sample required;
- Development of sample registry methods for use on a micro-scale to allow for application of multiple techniques to one sample location;
- Development of techniques for applying calorimetry to small sample size;
- Compilation of a database of likely terrestrial microbial contaminants;
- Development of search logic algorithms that consider the complexity of the sample;
- Investigation of the effects of a martian atmosphere versus an inert atmosphere on proposed methods and technologies; and
- Continued optimization of cleaning techniques and clean room technology

PLENARY DISCUSSION: WHAT IF LIFE IS DETECTED?

Introduction

During a one-hour session on the final day of the Workshop, the participants explored the implications of actually discovering life in the sample. The plenary discussion, chaired by Margaret Race, focused on the question: "If life is detected in the sample (other than confirmed terrestrial contamination), what are the next steps?"

In the Workshop Series to date, participants have concentrated on the science, methods, instruments, and facilities needed to conduct rigorous analyses in the search for life and biohazards within the sample. Their working assumptions all along have been consistent with the recommendations of the SSB: if no life is detected and sample materials are determined not to be biohazardous, samples may be released from quarantine for controlled distribution to the scientific community. Even if all life detection and biohazard tests are negative,[27] decisions about what is done with sample materials will likely be made only after review by an appropriate international scientific oversight committee at the Sample Receiving Facility (SRF) in consultation with NASA's Planetary Protection Officer and other responsible officials. Based on experiences during the ALH-84001 debate about possible fossil life in a martian meteorite, the decision to release pristine materials from quarantine will probably be complicated by the difficulties of distinguishing false positives and terrestrial contamination from possible martian entities.

The situation will be dramatically different if life forms of non-terrestrial origin are detected and verified in sample materials. The discovery of extraterrestrial life would likely dictate continued containment for an indefinite period of time and the need to reserve sample materials for comprehensive characterization and further study. Already, others in the astrobiology community have begun to ponder the societal, legal, ethical, theological, and non-scientific implications of such a profound discovery. At this time, it is appropriate to anticipate the ramifications of the detection of extraterrestrial life, not only the scientific questions but also those that relate to the protocol testing and the operations of the SRF *per se*.

Participants were asked to focus on the kinds of questions and issues that might arise if martian life were discovered and what specific steps in the protocol and beyond would need attention. Rather than develop specific recommendations at this time, the group focused on identifying issues that need further discussion in advance of sample return. In the open discussion, numerous issues were identified that fall into three broad categories: Science and Testing, Facility and Technological, and Policy and Administrative, as discussed below.

Science and Testing Issues Related to Discovery

Consistent with the SSB recommendation, the participants advocated that no materials should be released from maximum containment if life is discovered in any sample material. In addition, testing should be stopped until a scientific oversight committee is able to review the adequacy of the protocol and provisions for containment. Having a fully constituted committee in place with

27 The word "negative" is used in this context to mean that no evidence of any life form or biohazard is detected; conversely a "positive" result is when a life form or biohazard *is* detected.

appropriate multidisciplinary expertise will be essential to accomplish the necessary data interpretation and review in the event that evidence of non-terrestrial life is detected.

While it is impossible to stipulate a specific review process at this time, the group identified the kinds of concerns that will need to be addressed within the protocol, especially those with direct bearing on scientific study and safety. Key areas of concern included: sample preparation methods and selection of sub-samples; amounts of materials to be used for future tests; the roles of non-destructive versus destructive tests; the sequence of tests in the protocol; conditions for allowing materials to be tested at facilities outside the SRF; the procedures for sterilizing and releasing selected sub-samples; verification of whether and how the organism interacts with the laboratory and biocontainment materials; and review of recommended conditions for banking, storage and curation of materials. Decisions about changes in the protocol are likely to depend on when in the protocol sequence life is found (e.g., early versus later; in life detection tests or via biohazard assays, etc.) and where it is found (e.g., in gloveboxes at the SRC; in geological materials, in tissue culture or infectivity tests; in monitoring or containment materials such as HEPA filters; at some facility outside the SRF where contained materials or sterilized sub-samples were sent, etc.). The review should also consider the nature of evidence for martian life (active versus dormant; chemical signature versus structural evidence; biomaterials or pieces; fossilized; or not carbon based.) In addition, discussion will be needed on what criteria should be used to determine if the biological entity is 'alive' or 'dead.'

If and when life is detected, emphasis will necessarily shift to searching for evidence of something more in sample materials. It will be advisable to scan for additional evidence with more than one search method, preferably using a combination of methods that emphasize broad, quick, and non-destructive tests. In the scans it will be important to determine if life is associated with only certain parts or features in the sample, and what implications the 'within-sample location' has on further testing and handling (e.g., if evidence is found in rock, should dust and soils also be considered contaminated? Would a filtered gas samples still be cleared for controlled distribution?) For efficient testing and minimal use of sample materials, a system for exact tracking and registration of finds within samples will be critical.

Following the initial scans, regardless of how few or how many individuals or colonies are found, the emphasis will undoubtedly shift from searching to characterization of the life form. In addition to isolating cells or entities, it will be important to understand what culture and environmental conditions are required to grow more of them for study in the lab, and what precautions are needed in the process. It will also be important to determine the sterilization doses necessary to kill organisms. Demonstration and validation of sterilization and containment techniques will be needed prior to proceeding with any contained transportation outside the SRF. This demonstration of methods will be essential for allowing the sample materials to be studied at the best facilities with the necessary expertise if not available at the SRF.

While detection of life would undoubtedly lead to an emphasis on further biological study, it will also be important to review the protocol for recommended modifications in physical, geological, and chemical tests of sample materials. In addition to reviewing the recommended flow of samples and sequences of tests, it may be necessary to add or delete tests based on what has been found. Moreover, it will also be necessary to review plans and procedures for deciding what

kinds of physical, chemical, and geological tests may be conducted on pristine versus sterilized sub-samples, either at the SRF or elsewhere.

Facility and Technological Issues

During the anticipated review of the protocol, questions about the adequacy of the SRF must also be addressed. Among the questions that will be important are: Will there be a need to add equipment or change operations in any way because of what has been found? Will there be need for facility upgrading of any type? Will there be an expectation for scientists and the public to 'watch' laboratory operations in real time, perhaps requiring the use of web cams or telepresence? Would there be a need for increased use of robotic operations? Are monitoring and emergency plans at the SRF sufficient? What is the advisability of having sample materials at more than one facility? If sample materials have been distributed to more than one facility, what should be done if evidence for life is detected at one location, but not another? What are the implications of finding the martian life outside the SRF? What is the 'worst case' scenario which could arise and how will it be planned for, handled, by whom, etc.?

Policy and Administrative Issues

If martian life is detected and a review of the protocol is required by a special committee, both short- and long-term policy issues will also need to be addressed. In the short term, the concerns generally relate to procedures and communications, and in the long term, to future large-scale plans.

Initially, one of the most important issues to discuss will be that of access to sample materials. The group identified a number of pressing concerns including the following questions: What policies should apply to controlled distribution of sample materials if and when martian life is detected? What would be the respective roles of the Curation and Analysis Planning Team for Extraterrestrial Materials (CAPTEM) committee and the SRF scientific advisory committee in making decisions about access to sample materials for further study? It might be advisable to have a pre-designated team of on-call experts to come to the SRF if life is detected. If so, what would be the appropriate balance of scientific disciplines, skills, agency affiliation, and nationality to be represented on the team? Given the significance of the discovery, perhaps it would also be advisable to consider having a historian, ethicist, or member of the general public on the team as well.

The recent experience with martian meteorite ALH-84001 can serve as a helpful guide to anticipating some of the problems that may arise beyond science questions *per se*. To the extent possible, it will be advisable to consider well in advance how to respond to pressure from scientists and scientific societies with interests in studying or having access to sample materials. Likewise, prior to sample return, it is advisable to discuss who will publish research findings when they are made. Questions about where and when scientific results will be published and under what authorship and review conditions must be addressed well in advance to avoid the appearance that an elite group of scientists exists with unfair access to this significant discovery. Announcing the discovery of extraterrestrial life would almost certainly generate a scientific controversy of immense magnitude. Again, using the martian meteorite experience for instructive purposes, it will be important to anticipate how to handle the differences of expert opinion and

public uncertainty that will arise in the weeks, months and even years after the announcement. Using lessons learned from the ALH-84001 situation and other relevant scientific controversies, it will be advisable to develop an organized communication plan well in advance to avoid a frenzied, reactive mode of communications with government officials, the scientific community, the mass media, and the public. In short, scientists and NASA should plan for contingencies in order to avoid the impression that decisions are being made in crisis mode. Any plan that is developed should avoid a NASA-centric focus by including other government agencies, international partners, and organizations as appropriate (e.g., World Health Organization, United Nations). To date, as NASA has proceeded in developing its plans for sample return handling, no government agencies have expressed great concern about the prospect of discovering extraterrestrial life. A verifiable detection of extraterrestrial life may well change the responses of a long list of domestic and international agencies whose mandates cover environmental and health and safety issues (e.g., CDC, EPA, USDA, NIH, WHO, and other relevant international agencies). In addition, although unlikely, the presence or controlled distribution of verified extraterrestrial life might prompt questions related to national or international security, or eco-terrorism, resulting in the possible involvement of other agencies (e.g. Department of Defense, Department of State, United Nations, etc.) Thus, it will be appropriate to plan for a wide range of questions and the possible involvement of many different agencies ahead of time.

It will also be advisable to anticipate the kinds of questions the public might ask (e.g., What is it? Is it dangerous? Can it escape? Is it appropriate to have it on Earth? etc.) and disclose information early and often to address their concerns, whether scientific or non-scientific. Following the initial discovery, status reports to government officials, the scientific community, the mass media, and the public will undoubtedly be needed. It will be advisable to discuss in advance the advantages and disadvantages of making subsequent research announcements in real time or with some time lag to allow for needed scientific validation.

In the long term, the discovery of extraterrestrial life, whether *in situ* or within returned sample materials, would also have implications beyond science and the SRF *per se*. In the period between now and the first sample return, it is unclear what impact other precursor missions could have on sample return and containment plans. Evidence of possible oases, water, fossils, or microbial life on Mars would most certainly trigger a review of sample return and protocol plans for the first sample return, as well as for subsequent robotic missions and human missions as well. If life is found within the samples on Earth, questions may arise about ownership of the data or the entity itself, and perhaps about 'patentability,' if there are features of interest. Considering that international partners will be involved in sample return, these and other legal questions may be complicated by differences in laws in the U.S. and elsewhere.

Beyond the implications for science, policy and future missions, the discovery of life would have profound significance in societal, ethical, theological and other realms. Many of the ramifications have been discussed in the context of NASA's ongoing Astrobiology research program. Clearly, in anticipation of a possible discovery, it will be especially important to educate a multidisciplinary cadre of scientists and students to be prepared to grapple with the many complicated issues ahead.

APPENDIX A1:
WORKSHOP SERIES BASIC ASSUMPTIONS

The Workshop Series was designed to touch on a variety of questions in pursuit of the stated objective, such as: "What types/categories of tests (e.g., biohazard, life detection) should be performed upon the samples? What criteria must be satisfied to demonstrate that the samples do not present a biohazard? What constitutes a representative sample to be tested? What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what physical/chemical analyses are required to complement biochemical or biological screening of sample material? Which analyses must be done within containment and which can be accomplished using sterilized material outside of containment? What facility capabilities are required to complete the protocol? What is the minimum amount of time required to complete a hazard-determination protocol? By what process should the protocol be modified to accommodate new technologies that may be brought to practice in the coming years (i.e., from the time that a sample receiving facility would be operational through the subsequent return of the first martian samples?)

To keep the Workshops focused, a set of basic assumptions were provided to guide and constrain deliberations; these assumptions were:

1. Regardless of which mission architecture is eventually selected, samples will be returned from martian sites which were selected based on findings and data from the Mars Surveyor program missions.
2. Samples will be returned sometime in the next decade.
3. Samples will not be sterilized prior to return to Earth.
4. When the Sample Return Canister (SRC) is returned to Earth, it will be opened only in a Sample Receiving Facility (SRF) where samples will undergo rigorous testing under containment and quarantine prior to any controlled distribution ('release') for scientific study.
5. The amount of sample to be returned in a SRC is anticipated to be 500-1000 grams.
6. The sample will likely be a mixture of types including rock cores, pebbles, soil, and atmospheric gases.
7. The amount of sample used to determine if biohazards are present must be the minimum amount necessary.
8. Samples must be handled and processed in such a way as to prevent terrestrial (chemical or biological) contamination.
9. Strict containment of un-sterilized samples will be maintained until quarantine testing for biohazards and life detection is accomplished. Sub-samples of selected materials may be allowed outside containment only if they are sterilized first.
10. The SRF will have the capability to accomplish effective sterilization of sub-samples as needed.
11. The SRF will be operational two years before samples are returned to Earth.
12. The primary objective of the SRF and protocols is to determine whether or not the returned samples constitute a threat to the Earth's biosphere and populations (not science study *per se*) and to contain them until this determination is made.

APPENDIX A2:
WORKSHOP 3: ISSUES AND TOPICS

(J. Schad, 03/15/01)

Over the next decade or so, NASA and its international partners are planning to engage in a Mars Sample Return mission to launch robotic missions to Mars with the objective of returning martian surface and sub-surface soil and rocks and atmospheric samples to Earth. The ultimate goal of these robotic missions is to enable research on the returned soil and rock samples, studies that are anticipated to provide a wealth of knowledge about the history of Mars and its environment. Before martian soil and rock samples can be distributed to the research community, the returned materials will initially be quarantined and examined in a proposed BSL-4 containment facility to assure that no putative martian microorganisms or attendant potential biohazards exist. During the initial quarantine, state-of-the-art life detection and biohazard testing of the returned martian samples will be conducted. Life detection, as defined here in regard to Mars sample return missions, is the detection of living organisms and/or materials that have been derived from living organisms that may be present in the sample.

Life detection methods must be both sensitive and comprehensive in order to preclude the untoward release of undetected putative martian biota outside the proposed BSL-4 containment facility when sub-samples are distributed. Moreover, the methods ultimately selected must be efficient and cost-effective to maximize life detection efforts and minimize ineffective procedures that waste sample material. Martian samples will present additional challenges inasmuch as conventional environmental studies conducted, heretofore, have not routinely focused on geological materials. In addition, applicable control methods and procedures must be developed that will distinguish unmistakable terrestrial contaminants from putative martian biota. Geochemical analyses must be performed on sub-samples subjected to life detection assays in order to define the inherent properties of the materials returned. It will also be necessary to delineate and prioritize the methods deemed most applicable to detect putative martian biota.

NASA has previously conducted three workshops in the Mars Sample Handling Protocol (MSHP) Workshop Series: Workshop 1 (convened March 2000) developed an overview and conceptual approach of Mars sample return issues; Workshop 2 (convened October 2000) emphasized biohazard testing and briefly discussed how life detection might help in assessing biohazards; and Workshop 2a (convened November 2000) Focused upon effective sterilization procedures and practices that may be applied to martian soil and rock sub-samples before controlled distribution of any sample materials out of the BSL-4 containment facility.

Workshop 3 of the MSHP Series will focus on defining the preliminary life detection protocol. This will, in conjunction with the results of the other workshops mentioned above, form the basis for the ultimate selection of applicable methods that will be employed to attempt to detect putative martian life and assess potential biohazards. Participants at this life detection workshop will be divided into sub-groups by their scientific disciplines and research experience(s) to explore methods as well as to define testing approaches and priorities. Inasmuch as most terrestrial microbes cannot be cultured by conventional methods, participants must deliberate the applicability of sensitive chemical and cellular detection approaches. The life detection workshop will culminate in the development of an interim

report that will be taken into account later by NASA and its partners in the formulation and approval of a final quarantine protocol. For brevity's sake, this document shall use "NASA" as the operator of the quarantine facility and the enactor of the protocol, although it is assumed that a future planetary protection protocol team will, in fact, include international participants.

Issues to be considered by workshop participants include:

- If putative martian life exists, it may somewhat resemble terrestrial life given the exchange of meteorites and potentially microbes between Earth and Mars within the inner solar system over the past 4 billion years. While divergent evolution on two worlds would likely evoke inherent differences, what fundamental, unifying properties of life as we know it should be tested, employing chemical and cellular assays that are usually used to monitor terrestrial biological activity?

- Putative martian biota may be quite different from terrestrial life, presenting morphological organization and chemical properties for which we have little or no points of reference. If so, what fundamental properties and organization (e.g., chemical changes or anomalies that suggest metabolic activity) should be taken into account to maximize detection of putative martian life and distinguish it from false positive evidence?

- Many terrestrial species survive through stages of dormancy during different life cycle stages, as well as over a range of environmental conditions. Putative martian life may likewise exhibit stages of dormancy or sporulation that could limit analytical sensitivity and overt detection. What chemical and cellular methods to detect dormant life-forms should be considered given the potential limits imposed to assay sensitivity?

- Only a portion of the returned martian sample will be tested under the quarantine protocol, thereby preserving the remainder of the sample for subsequent research. Consequently, all life detection testing would be performed on 'representative sub-samples.' What geochemical and geophysical properties of the entire returned sample must be taken into account to select representative sub-samples? What are the final criteria for sub-sample selection and preparation? Are there other procedures and methods[28] that have potential applications to Mars samples and at the same time preserve the properties of soil and rocks for future planetary geology research? Should NASA invest in research into the potential applications of these emerging procedures?

- In light of anticipated difficulties in the detection of putative martian life (e.g., potentially dormant forms or putative microbes existing as a low biomass in the sample), how many representative

28. For example, X-ray tomography is an emerging method that may have potential applications for life detection analyses of the entire sample returned from Mars without disturbing the properties of soil and rocks that are reserved for planetary geology research. C.J. Buckley (Kings College, London) recently noted that current advances in the emergence of high-resolution X-ray optics and X-ray sources will create over the next few years "a considerable expansion in the use of X-ray microscopy to tackle problems in the biological and material sciences." However, the method requires a separate synchrotron facility and equipment to generate the scanning X-ray beamline (outside the proposed Mars quarantine protocol BSL-4 containment facility). [Buckley, C.J., "X-Ray Microscopy," In: *Structure and Dynamics of Biomolecules: Neutron and Synchrotron Radiation for Condensed Matter*. Edited by E. Fanchon (Institute de Biologic Structurale Jean-Pierre Ebel, Grenoble, FR) et al., Oxford University Press (2000).]

sub-samples should be tested in the quarantine protocol? What is the acceptable margin of error that will assure NASA has not missed putative martian life's detection?

- At prior quarantine protocol workshops, several participants with expertise in terrestrial microbial life detection have emphasized a high priority requirement for sensitive chemical analyses in order to detect a low biomass or dormant putative martian biota. In addition to fundamental elemental chemical analysis of representative sub-samples, what chemical methods should receive a high priority? What methods and procedures should be considered to reduce the margin of error? What type and number of controls will be needed to definitively distinguish potential terrestrial contamination?

- If putative martian microbes exhibit recognizable morphological characteristics, what cell biology methods (e.g., electron microscopy, flow cytometry, etc.) provide the highest-ranking priorities for life detection, even though putative martian biota may exist in a low biomass or in dormant forms? What methods and procedures should be considered to reduce the margin of error? What controls will be used to definitively distinguish potential terrestrial contamination?

And for final discussion: If life is detected in the returned martian sample(s) (other than confirmed terrestrial contamination), what are the next steps?

APPENDIX B:
WORKSHOP 3 AGENDA

Day 1: Monday 19 March 2001

9:00 a.m.	Welcome and Logistics
9:10 a.m.	Introduction to the MSHP Workshop Series (J. Rummel, NASA Headquarters)
9:20 a.m.	Overview of Mars Program (J. Rummel, NASA Headquarters)
9:40 a.m.	Mars Sample Return Mission Planning (D. Beaty, NASA JPL)
10:00 a.m.	Break
10:30 a.m.	Report of LIFARS Workshop (K. Nealson, NASA JPL and D. Blake, NASA Ames)
10:40 a.m.	Report on NAS Life Detection Workshop (J. Baross, University of Washington)
10:45 a.m.	French Planning for Mars Missions (M. Viso, CNES)
11:00 a.m.	Summary of MSHP Workshops 1 and 2 (M. Race, SETI Institute and G. Kovacs, Stanford University)
11:45 a.m.	Objectives of MSHP Workshop 3 (J. Rummel, NASA Headquarters)
Noon	Define Day 1 Sub-group Charters and Members – SG 1A: Unifying Properties of Life (K. Nealson, Chairperson) – SG 1B: Morphological Organization and Chemical Properties (J. Baross, Chairperson) – SG 1C: Geochemical and Geophysical Properties (D. Blake, Chairperson)
12:30 p.m.	Lunch
1:30 p.m.	3 Sub-groups break out for individual discussions (all afternoon)
3:30 p.m.	Break
5:30 p.m.	Adjourn

Day 2: Tuesday 20 March 2001

8:30 a.m.	Plenary reports from Day 1 Sub-groups 1A, 1B, and 1C
10:30 a.m.	Break
10:50 a.m.	Define Day 2 Sub-group Charters and Members – SG 2A; Chemical Methods (J.L. Bada, Chairperson) – SG 2B: Cell Biology Methods (N. Wainwright, Chairperson)
11:00 a.m.	2 Sub-groups break out for individual discussions
12:30 p.m.	Lunch
1:30 p.m.	2 Sub-groups continue individual discussions (all afternoon)
3:30 p.m.	Break
5:30 p.m.	Adjourn

Day 3: Wednesday 21 March 2001

8:30 a.m.	Plenary reports from Day 2 Sub-groups 2A and 2B
10:30 a.m.	Break
10:50 a.m.	Open discussion on the question: If life is detected in the sample (other than confirmed terrestrial contamination), what are the next steps? (Margaret Race, Chairperson)
12:00 p.m.	Workshop wrap-up and conclusion
12:30 p.m.	Adjourn

APPENDIX C1:
WORKSHOP 3 PARTICIPANTS' AREA(S) OF EXPERTISE

Name	Affiliation	Area(s) of Expertise
Acevedo, Sara E.	SETI Institute	(Workshop Planning Committee Member)
Allen, Carl	NASA Johnson Space Center	Sample handling and curation; physical/Earth and planetary sciences.
Bada, Jeffrey L.	Professor, Marine Chemistry, Scripps Inst. of Oceanography	Structure, Stability, and Evolution of Proteins; Life Detection
Baross, John	School of Oceanography, University of Washington	Deep-sea microbiology, etc.
Beaty, David	NASA Jet Propulsion Laboratory	(Workshop Observer)
Bibring, Jean-Pierre	IAS, France	Planetology; Sample handling; Curation facility
Blake, David	NASA Ames Research Center	Microanalytical examination of extraterrestrial organic samples
Briggs, Geoffrey	NASA Ames Research Center	(Workshop Observer)
DeVincenzi, Donald	NASA Ames Research Center	(Workshop Planning Committee Member)
Ferry, J. Gregory	Pennsylvania State University	Functional genomics and transcription regulation in Archaea
Fogel, Marilyn	Carnegie Inst. of Washington	Stable Isotope Biogeochemistry
Grange, Jacques	Lab de Haute Securite P4 Jean Merieux	Responsible for the MERIEUX Biosfaety Level 4 facility; virology.
Kovacs, Gregory T.A.	Electrical Engineering, Stanford University	Biodefense; biohazard testing; cellular and molecular genetic mechanisms in pathogenesis.
Lambert, Joseph B.	Department of Chemistry, Northwestern University	Silicon Polymer Chemistry
Lindstrom, David	NASA Johnson Space Center	(Workshop Observer)
Mustin, Christian	Centre de Pédologie Biologique	Geologist and physicochemist; biochemical reactivity of microorganism-mineral interfaces.
Nealson, Kenneth	NASA Jet Propulsion Laboratory	Post-Viking microbiology/environmental microbiology; life detection
Nicholaides III, John J.	Soil Science Society of America	Soil Chemistry
Pardee, Arthur B.	Dana Farber Cancer Institute, Harvard University	Molecular evolution; cell cycle control; cancer etiology.
Race, Margaret	SETI Institute	(Workshop Planning Committee Member)
Relman, David A.	Dept. of Microbiology and Immunology, Stanford University	Microbial detection methods for unrecognized organisms; life detection
Rummel, John	Planetary Protection Officer, NASA Headquarters	(Workshop Planning Committee Member)
Schad, Jack	NASA Headquarters	(Workshop Planning Committee Member)
Singer, Michael J.	University of California, Davis	Professor of Soil Science and Soil Resources; Soil toxicology and the influence of climate on soil formation
Sogin, Mitchell L.	Biology and Evolution, Marine Biological Laboratory	Comparative molecular biology and evolution; life detection

Name	Affiliation	Area(s) of Expertise
Sourdive, David J.D.	Centre d'Etudes du Bouchet	Viral immunology, arenaviruses; high sensitivity detection and identification of potentially hazardous microorganisms.
Stabekis, Pericles D.	Lockheed-Martin	(Workshop Planning Committee Member)
Steele, Andrew	Astrobiology Group, University of Portsmouth, U.K.	Microbiology
Treiman, Alan H.	Lunar and Planetary Institute	Geology; physical/earth and planetary sciences
Viso, Michel	Centré National d'Etudies Spatiale (CNES)	Radionuclides in biology, applied medical statistics, animal and comparative immunology, domestic animal nutrition
Wainwright, Norman	Molecular Biology, Marine Biological Laboratory	Comparative molecular biology and evolution; life detection
Wali, Mohan K.	The Ohio State University	Ecology; soil science, environmental policy; Former Director, School of Natural Resources and Associate Dean, College of Agriculture

APPENDIX C2:
WORKSHOP 3 PARTICIPANTS' ROSTER

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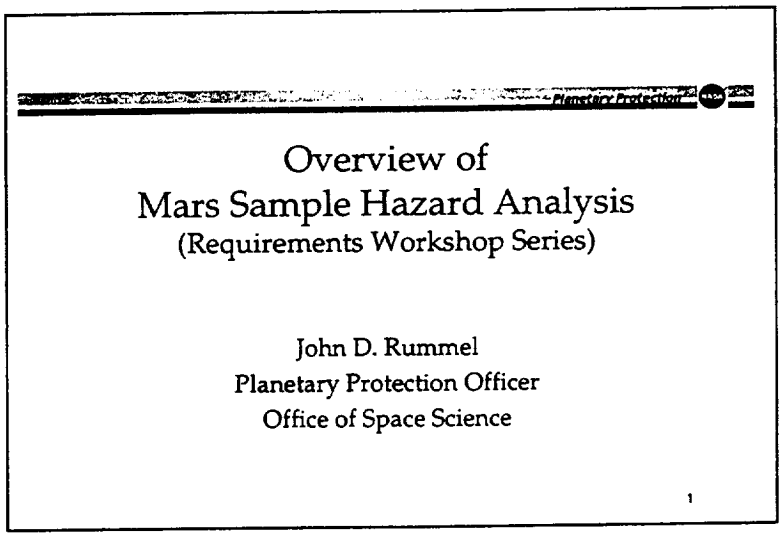
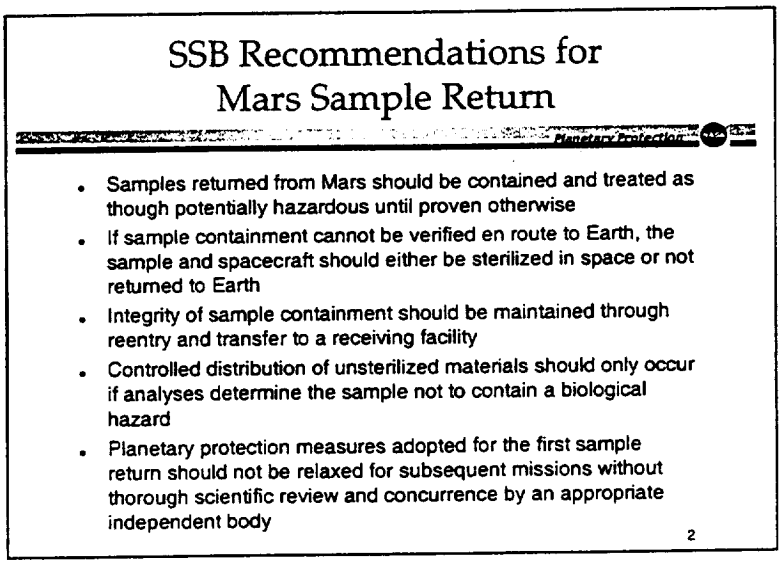
APPENDIX D:
BACKGROUND TUTORIALS

Introduction to Mars Sample Handling Workshop Series: Overview of Mars Sample Hazard Analysis

John D. Rummel, NASA Headquarters





Planning for Sample Hazard Analysis

Protocol Development Workshops

- Major question: What are required steps to meet the NRC recommendation that, "rigorous analyses determine that the materials do not contain a biological hazard," and "returned samples should be considered potentially hazardous until they have been reasonably demonstrated to be nonhazardous"
- Plan: A series of workshops will be organized to assess the requirements for sample hazard testing and subsequent release, specify the tests necessary to show that a biological hazard is not present in the sample
- Action: Develop a recommended list of comprehensive tests, and their sequential order, that may be performed to fulfill the NRC recommendations in a manner acceptable to biomedical scientists and regulatory agencies

3

Sample Hazard Analysis Assumptions

- The initial Mars Sample Return (MSR) missions will be launched in the first decade of the 21st Century, and will return samples to Earth no earlier than 2009.
- The missions will return samples from sites selected on basis of data to be returned from previous Mars Surveyor program missions.
- The samples will not be sterilized prior to return to Earth.
- Up to two separate sample return canisters (SRCs) will be returned to Earth in the initial mission. The SRCs will be opened only in a receiving facility.
- The amount of sample to be returned in each SRC is anticipated to be 500-1000 grams.
- The sample will likely be a mixture of types including rock cores, pebbles, soil, and atmospheric gases.
- The amount of sample used to determine if biohazards are present must be the minimum necessary.

4

Sample Hazard Analysis Assumptions

(cont.)

- Samples must be handled and processed in such a way as to prevent terrestrial chemical or biological contamination.
- Strict containment of unsterilized samples will be maintained until quarantine testing for biohazards and life detection is accomplished. Sub-samples of selected returned materials may be allowed outside containment only if they are sterilized first.
- The receiving facility will have the capability to accomplish effective sterilization of sub-samples as needed.
- The receiving facility will be operational two years before samples are returned to Earth.
- The primary objective of the laboratory and protocols is to determine whether or not the returned samples constitute a threat to Earth's biosphere and population (not science study *per se*) and to contain them until this determination is made.

5

Workshop Plan

Planetary Protection

- Workshop I
  - » March 20-22, 2000, Bethesda, Maryland USA
  - » Objective: Establish the context, overall approach and product(s) of the workshop series; outline a preliminary, comprehensive, beginning-to-end scenario for a Mars sample handling protocol and timeline to determine if the samples contain a biological hazard.
- Workshop II
  - » October 2000, East Coast USA
  - » Objective: Develop MSR PP biohazard determination protocols and timeline as a refinement of the scenario developed in Workshop I. Specify in detail the preferred methodologies for biohazard determination that will comprise a major portion of the protocol.

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Workshop Plan (cont.)

Planetary Protection

- Workshop III
  - » February 2001, East Coast or California USA
  - » Refine life detection protocol as a refinement of Workshop I and the NRC Life Detection Workshop. Specify the preferred methodologies for life detection that will comprise a major portion of the protocol.
- Workshop IV
  - » April 2001, East Coast USA
  - » Objectives:
    - – Finalize detailed requirements to be met by any protocol and process
    - – Finalize an acceptable MSR sample hazard determination protocol and the maintenance and oversight process for modification/updating of protocol by the Mars sample handling project personnel
    - – Integrate and finalize sample handling requirements and methodologies into a protocol on which to base facility cost/sizing projections; outline final report findings and recommendations.

7

Questions/Issues: Workshop 1

Planetary Protection

- What types/categories of tests (biohazard determination, life detection) should be performed upon the samples? What scientific controls should be implemented? What preliminary characterization information is required for these tests to be implemented?
  - » Identify amounts of sample needed for these tests.
- How will representative sub-samples for all tests be selected?
- How will the nature of the sample (i.e., rocks, soil, cores, etc.) affect the tests chosen?
- In what sequence shall the relevant testing be performed?
- What tests can be performed on sterilized samples outside of containment?

8

Questions/Issues: Workshop 1 (cont.)

Planetary Protection

- What is the range of relevant test results and interpretations that might cause concern?
- What are the criteria for release of samples from containment?
- Assess the pros and cons of using multiple containment facilities to determine if the samples contain a biological hazard.

9

Questions/Issues: Workshops 2 & 3

Planetary Protection

- In what sequence will the specific characterization, biohazard determination, and life detection analyses be performed?
- What are the necessary, sufficient, and relevant biohazard determination and life detection tests to be performed?
- What are the various possible interpretations of results from the suite of biohazard determination and life detection analyses?
- Assess the extent to which the detailed tests meet the objectives of other interested parties (e.g., regulatory agencies, international partners, etc.)

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Questions/Issues: Workshop 4

Planetary Protection

- Integrate the detailed methodologies for biohazard determination and life detection into a recommended protocol and timeline.
- Assess how the recommended analyses will satisfy the criteria for release of samples from containment.
- How will advances in methods/technologies in the coming years be incorporated into the recommended protocol? How will the protocol be amended in the future up to the receipt of samples? How will this process be overseen/reviewed by Planetary Protection?
- What considerations of facilities, equipment, and personnel are important for implementing the recommended protocol?
- Develop outline of findings and recommendations for final report.

11

Planning for Sample Hazard Analysis

Planetary Protection

- Organizing committee, Chaired by NASA Planetary Protection Officer (with CNES participation)
- Senior-Level Oversight and Review Panel (~25 people) to advise the organizing committee on the planning, organization, participants, and conduct of the workshops (US and France)
  - Chosen for their abilities to address key scientific, biohazard evaluation and quarantine protocol issues associated with handling, characterizing, testing, and judging whether returned sample materials are in any way biohazardous, and when and whether they may be certified for controlled distribution outside containment and quarantine
  - Will provide peer review of the protocol, prior to its release for external review by appropriate groups outside of NASA
- Workshop participants (by invitation)

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Workshop Products

Planetary Protection

- Individual Workshops:
  - Summary of material analyzed (advance reading, handouts, subgroup reports, etc.)
  - Interim report of findings and recommendations prior to next workshop
  - Briefing package
- Final Workshop Series:
  - Final report of findings and recommendations, reviewed by Oversight and Review Committee
  - Briefing package suitable for presentation to advisory groups, regulatory agencies, scientific meetings, etc.
  - Recommendations in a form suitable for use as input for possible future announcements of opportunity soliciting proposals for Mars sample handling participants/capabilities.

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Planning for Sample Hazard Analysis

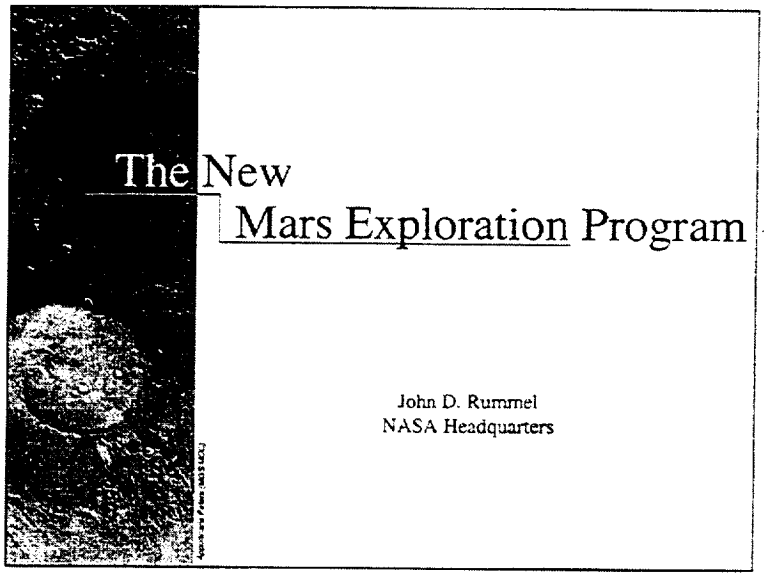
Planetary Protection

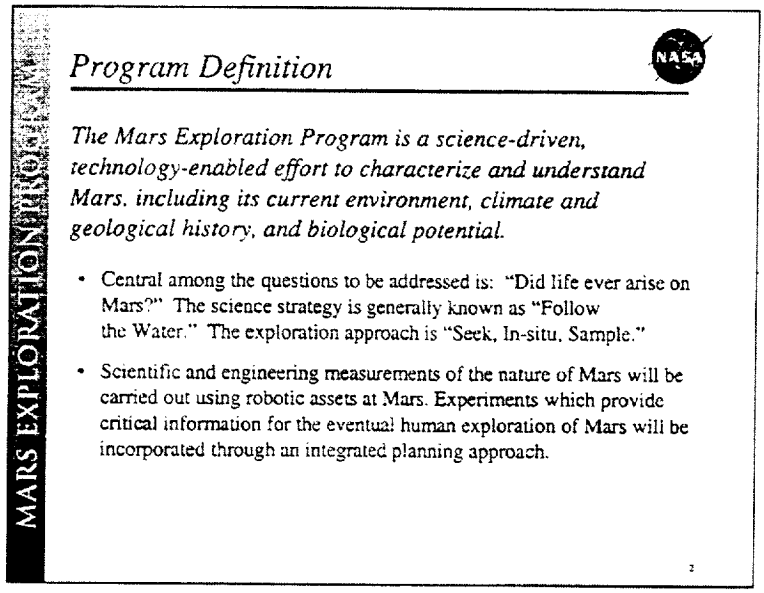
- Post-Workshop Tasks
  - Preparation of overall report and protocol details
  - Review by Oversight and Review Panel and revisions
  - Submit final document
  - Endorsement by NASA Advisory Council / Planetary Protection Advisory Committee; Parallel review by CNES, etc.
  - Dissemination of report to relevant audience(s) or Agencies
  - Approval by other Agencies, and availability for use in planning for activities in the Mars Receiving Facility, etc.

14

Overview of Mars Program

John D. Rummel, NASA Headquarters





MARS EXPLORATION

Program Development Strategies

Science

- Meet science priorities in goals, objectives & investigations
- Conduct science through "Seek, In-situ, Sample"
- Be responsive to new discoveries
  - Allow sufficient time between related missions to interpret data
- Conduct multiple Mars Sample Returns
- Conduct competed small scout missions ("Discovery" approach)

Technology

- Develop key technologies before committing to final design for major mission
- Allow phased introduction of the technology through precursor missions
- Emplace Telecommunications/Navigation infrastructure

Management

- Safety and Mission Success a first priority
- Continuing emphasis on program-level systems engineering
- Distributed Risks (redundant missions where appropriate, limit size/cost of payload on single launch vehicle)
- Open to International Participation
  - Where there is a clear mutual benefit
  - Consistent with program strategy
- Resilient to mission failures (NASA and International)
- Resilient to schedule slips of international elements

MARS EXPLORATION

Goals and Objectives of the Science-Driven Mars Exploration Program

Goal -- Life: Determine if life ever arose on Mars

- Determine if life exists today
- Determine if life existed on Mars in the past
- Assess the extent of prebiotic organic chemical evolution on Mars

Goal -- Climate

- Characterize Mars's present climate and climate processes
- Characterize Mars's ancient climate

Goal -- Geology

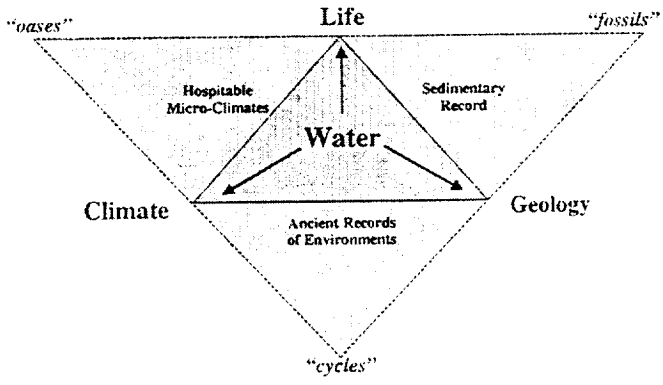
- Determine the geological processes that have resulted in formation of the Martian crust and surface
- Characterize the structure, dynamics and history of Mars interior

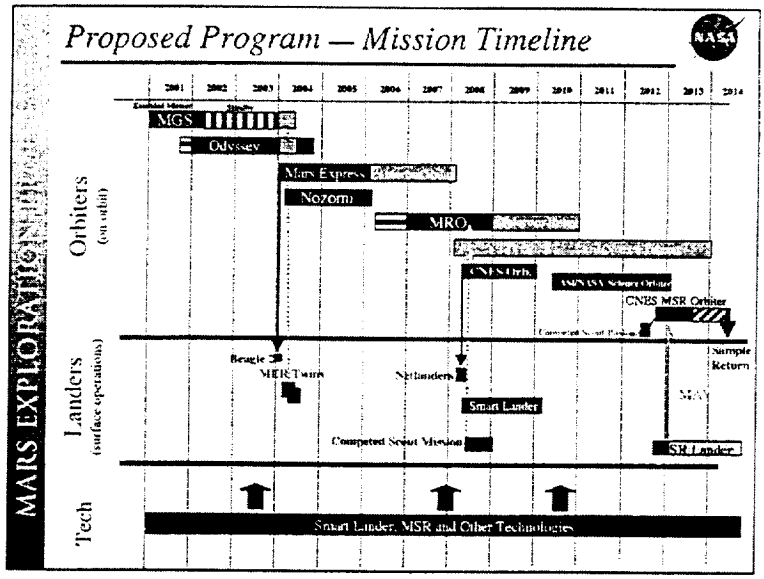
Goal -- Prepare for Human Exploration

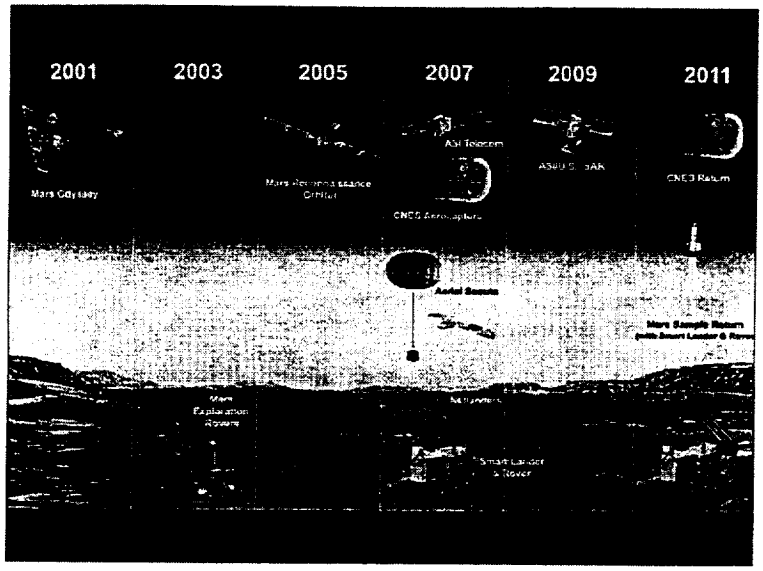
- Acquire Martian environmental data set (such as radiation)
- Conduct in-situ engineering/science demonstration
- Emplace infrastructure for future missions

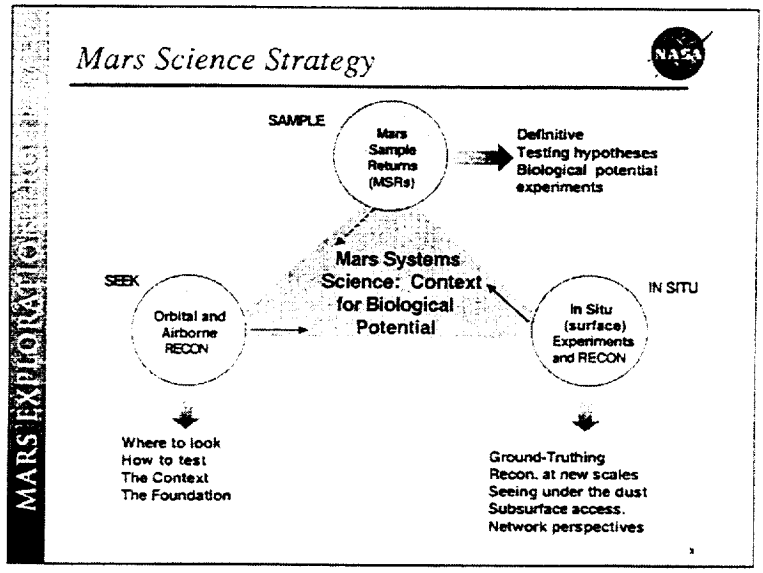
MARS EXPLORATION

Mars: A Systems Science Approach



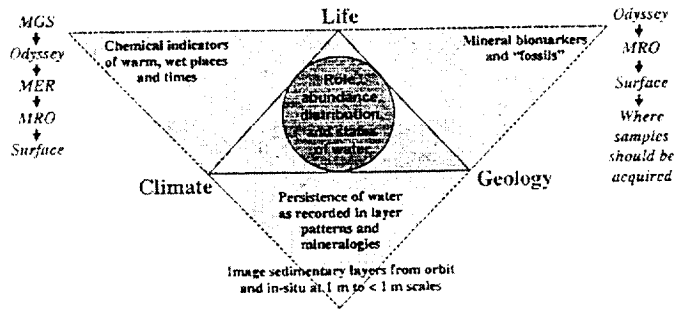

Proposed Program — Mission Timeline
MARS EXPLORATION
Orbiters
Landers
(surface operations)
Tech
MGS
Odyssey
Mars Express
Nozomi
MRO
CNES Orb.
CNES MSR Orbiter
Beagle 2
MER Twins
Netlanders
Smart Lander
Competed Scout Mission
MSR Lander
Sample Return
Smart Lander, MSR and Other Technologies


2001
2003
2005
2007
2009
2011
Mars Odyssey
CNES Return


Mars Science Strategy
MARS EXPLORATION
SAMPLE
Mars Sample Returns (MSRs)
Definitive
Testing hypotheses
Biological potential
experiments
Mars Systems Science: Context for Biological Potential
SEEK
Orbital and Airborne RECON
IN SITU
In Situ (surface) Experiments and RECON
Where to look
How to test
The Context
The Foundation
Ground-Truthing
Recon. at new scales
Seeing under the dust
Subsurface access.
Network perspectives

MARS EXPLORATION

New Mars Program Strategy: 2001 - 2010



- Where to go,
- What to look for at or just below the surface, and ultimately
- What to return to Earth

MARS EXPLORATION

Mars Technology Program: Technology Objectives

- Determine technology requirements for the Mars Program
- Assess the state of development of technologies important in Mars exploration
- Define, develop, validate and insert into flight missions the key technologies needed for Mars exploration
- Engage the best talents at JPL, NASA Centers, universities and industry in technology definition and development
- Develop partnerships with other technology sponsors

MARS EXPLORATION

Mars Technology Program: Technology Requirements

Mars '07 Lander

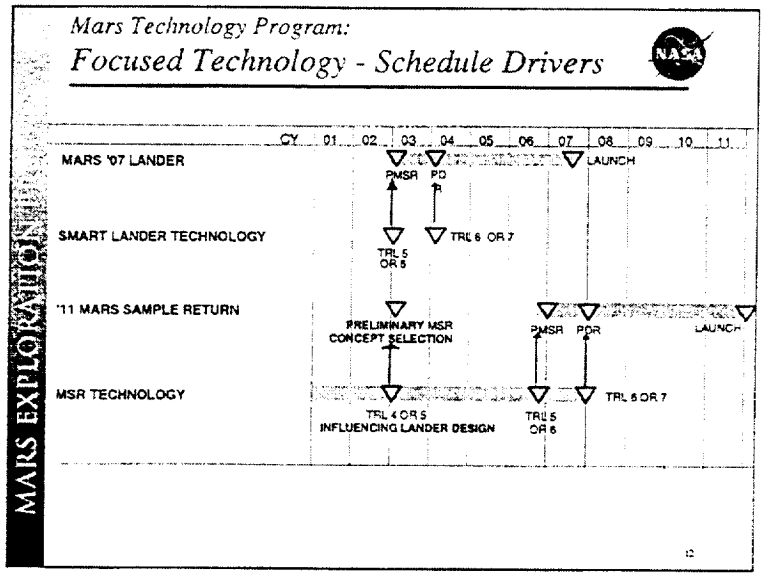
- Precision Landing - better than 5 km
- Hazard Avoidance - "eyes wide open" at landing
- Robust Landing - failsafe touchdown systems
- Long duration surface operations - 1-5 years
- Surface mobility - "Go To" capability with mobility of 5 to 10 km

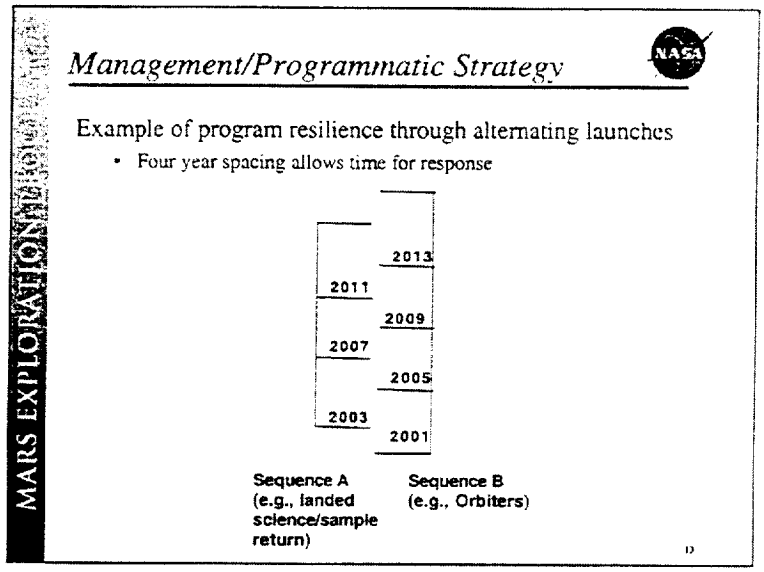
Mars '11 Sample Return

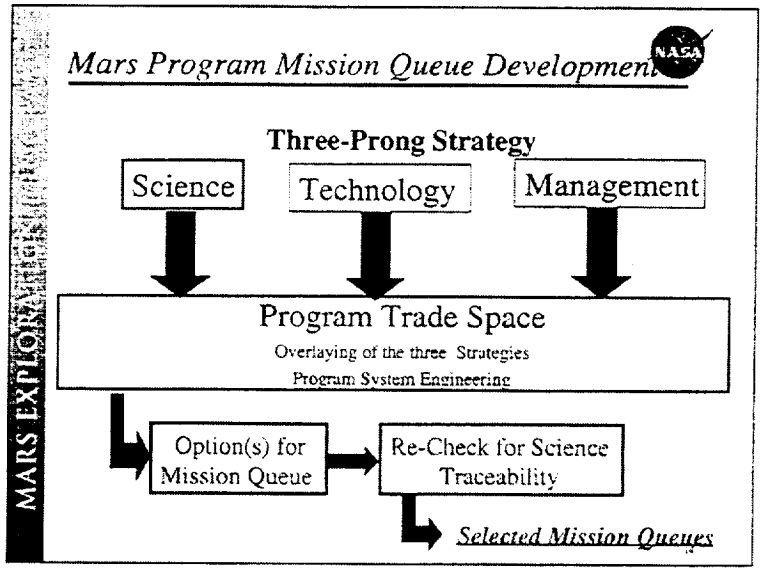
- Select and collect suitable samples
- Prevent biological contamination of sample with Earth organisms
- Launch sample from Mars surface towards Earth
- Track and rendezvous with sample canister
- Avoid back contamination of Earth by potential Mars organisms

Advanced Capabilities

- Mars Proximity Communications
- Aerocapture and space propulsion
- Deep Subsurface Exploration up to 400m
- Aerial Platforms with useful lifetimes
- Advanced In-situ sensors for detecting biological potential
- Autonomous exploration systems

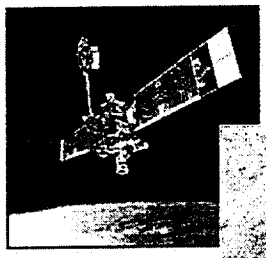

Mars Technology Program:
Focused Technology - Schedule Drivers
MARS EXPLORATION
CY 01 02 03 04 05 06 07 08 09 10 11
MARS '07 LANDER
PMSR
PDR
LAUNCH
SMART LANDER TECHNOLOGY
TRL 5 OR 6
TRL 6 OR 7
'11 MARS SAMPLE RETURN
PRELIMINARY MSR CONCEPT SELECTION
PMSR
PDR
LAUNCH
MSR TECHNOLOGY
TRL 4 OR 5 INFLUENCING LANDER DESIGN
TRL 5 OR 6
TRL 6 OR 7


Management/Programmatic Strategy
MARS EXPLORATION
Example of program resilience through alternating launches
• Four year spacing allows time for response
2013
2011
2009
2007
2005
2003
2001
Sequence A (e.g., landed science/sample return)
Sequence B (e.g., Orbiters)


Mars Program Mission Queue Development
MARS EXPLORATION
Three-Prong Strategy
Science
Technology
Management
Program Trade Space
Overlaying of the three Strategies
Program System Engineering
Option(s) for Mission Queue
Re-Check for Science Traceability
Selected Mission Queues

MARS EXPLORATION

Mars Global Surveyor



Prime Mission: April '99 - Feb. '01
Extended Mission Just Begun!!

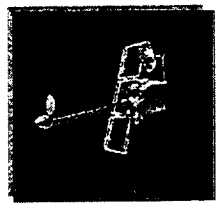
Major Instruments
Mars Orbiter Camera (MOC)
Laser Altimeter (MOLA)
Thermal Spectrometer (TES)

MARS EXPLORATION

2001 Mars Odyssey

Mission Description

- ***Launch - April 2001 / Mars Orbit Insertion - October 2001***
- ***Prime Mission - 76 days aerobraking, science mission through June 2004, relay mission through Oct. 2005***
- ***Science payload -***
  - *Thermal Emission Imaging System (THEMIS)*
  - *Gamma Ray Spectrometer (GRS)*
  - *Mars Radiation Environment Experiment (MARIE)*



Primary Objectives:

- THEMIS will map the mineralogy and morphology of the Martian surface using a high-resolution camera and a thermal infrared imaging spectrometer
- GRS will achieve global mapping of the elemental composition of the surface and determine the abundance of hydrogen in the shallow subsurface. GRS is a clone of the instrument lost with the Mars Observer mission.
- MARIE will describe aspects of the near-space radiation environment, especially the radiation risk to human explorers.
- Provide communications link for future Mars missions

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MARS EXPLORATION

2003 Twin Mars Exploration Rovers

Mission Description

- ***Launch - May/June 2003 / Mars Landing - Jan/Feb 2004***
- ***Prime Mission - 90 days surface operations, until late April 2004; could be continue longer depending on health of the rovers.***
- *"Athena" Science payload -*
  - *Panoramic Camera (Pancam)*
  - *Miniature Thermal Emission Spectrometer (Mini-TES)*
  - *Mössbauer Spectrometer*
  - *Alpha-Particle X-ray Spectrometer*
  - *Rock Abrasion Tool*
  - *Microscopic Imager*

Primary Objectives:

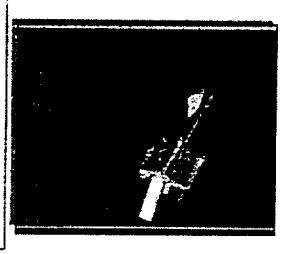
- Determine the aqueous, climatic, and geologic history of 2 sites on Mars where conditions may have been favorable to the preservation of evidence of pre-biotic or biotic processes.
- Identify hydrologic, hydrothermal, and other processes that have operated at each of the sites.
- Identify and investigate Martian rocks and soils that have the highest possible chance of preserving evidence of ancient environmental conditions associated with water and possible pre-biotic or biotic activity.
- Respond to other discoveries associated with rover-based surface exploration.

MARS EXPLORATION

2005 Mars Reconnaissance Orbiter

Mission Description

- *Launch - August 2005; enter Mars polar orbit*
- *Prime Mission - 3-5 years high resolution imaging and orbital characterization of Martian surface*
- *Science payloads under consideration*
  - *High resolution visible-near IR imaging spectroscopy (VNIRIS) (0.4 to 3 microns, 5 nm resolution, 50m/pixel)*
  - *High-resolution visible imaging (HRI) - (30-60 cm /pixel)*
  - *Infrared sounding and imaging of Martian atmosphere (MCO recovery)*
  - *Context imager*
  - *Other instruments under study*



Primary Objectives:

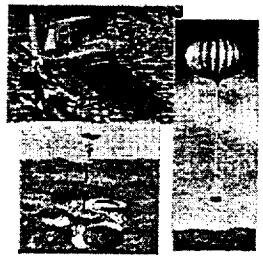
- Recover the Mars Climate Orbiter (MCO) MARCI and PMIRR investigation, emphasizing Mars volatiles (water) and climate history
- Search for mineralogic and morphologic evidence of water-related processes on a global basis
- Advance our understanding of the physical processes controlling the present transport, distribution and past evolution of water on Mars
- Conduct detailed study of regions of high scientific interest, including the Mars Global Surveyor discovery sites associated with "modern" water
- Characterize potential landing sites with regard to both scientific merit and landing safety
- 10 year extended mission telecommunication relay and navigation beacon

MARS EXPLORATION

Competed Scout Missions

Mission Description

- *Launch - Proposed 2007 (possible additional scout payload on CNES 2007 orbiter; additional scout missions proposed for accelerated program architecture)*
- *Goals for Mars Scout Missions*
  - *Incorporate into the Mars Exploration Program innovations in science, measurement systems, and mission concepts arising from researchers throughout the community.*
  - *Utilize the successful methodology proven by the Solar System Discovery Program*
  - *Engage continuously the university community in the end-to-end accomplishment of missions to Mars.*



Programmatic Objectives:

- Achieve the best possible, focused science investigations of Mars
- Create a capability within the program to respond rapidly to scientific discoveries
- Utilize a competitive process to select PI-led missions focused on science
- Rigorous review of mission concepts, implementation plans, management approach, and budget estimates
- Reach of the traditional program participants to science and hardware innovators for new and exciting missions

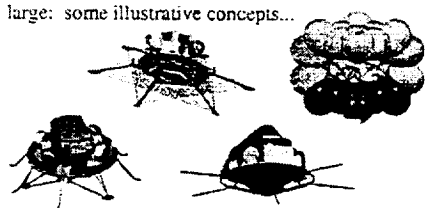
MARS EXPLORATION

Long-Life, Mobile, In-Situ Science

Mission Description

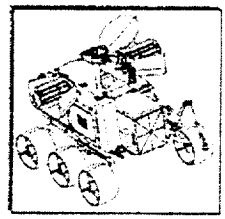
- *Launch - TBD*
- *Prime Mission - 1-2 years surface operations, could be continued longer depending on health of the rover.*
- *Long-duration power source*
- *Precision EDL and Active Hazard Avoidance*
- *Science payload to be competed*

Trade space for impact attenuation structure is large: some illustrative concepts...



Primary Objectives:

- In-situ science: Space Science & HEDS with international participation
- Utilize active hazard avoidance and precision landing
- Validate rover design and long-life operations for future MSR missions



MARS EXPLORATION

Example Vision for 2012-2020

Respond to discoveries in previous decade.

Expand surface access to:

- Network science
- Near subsurface H_2O (to 200m)
- Deep Subsurface (>200m)
- High latitudes.

Multiple Mars Sample Return missions

Long-term virtual presence for public engagement

21

MARS EXPLORATION

Mars Program Development Summary

- We have created a new, scientifically rich and publicly exciting Mars exploration campaign
- Combined strategies will address Mars' biological potential, mitigate risk, and bring the discovery process to all people
- Plans for beyond 2005 considered a "living document"
  - Mars will continue to surprise us, and technology will progress
- Mars Sample Return dominates the discussion and trade space
  - Advocated as a very high priority by majority of science community
  - Technology "rich" with numerous development challenges
  - Substantial investment required (orbiter and lander development, ELVs, Mars Ascent Vehicle, sample handling, technology, etc.)
- International participation is included
- Community consensus on "Mars Discovery" competitive opportunities for small"scout" missions (aerial platforms, small rovers, etc.)
- "Program systems engineering" during next 12 months will refine cost and implementation details

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MARS EXPLORATION

Mars Exploration Program

Questions?

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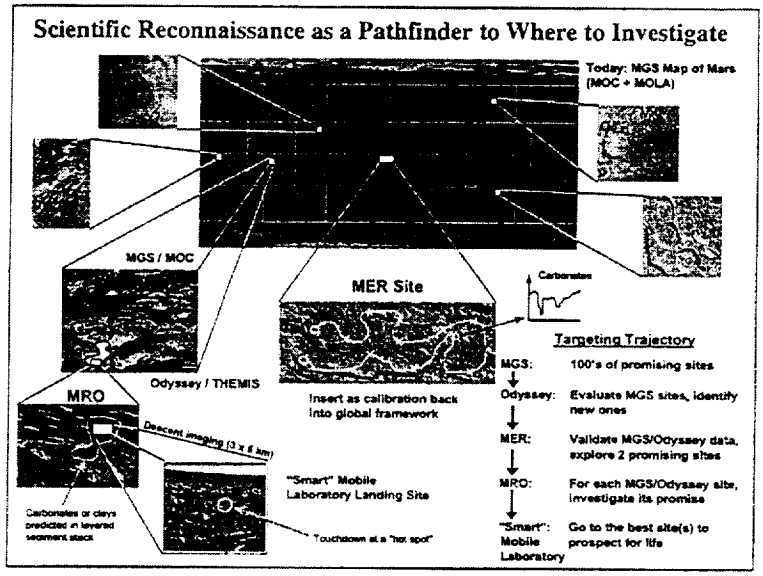
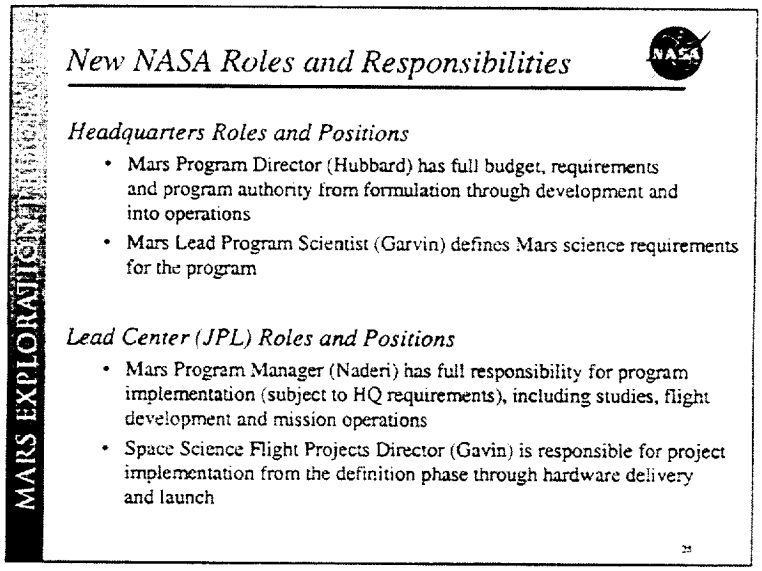
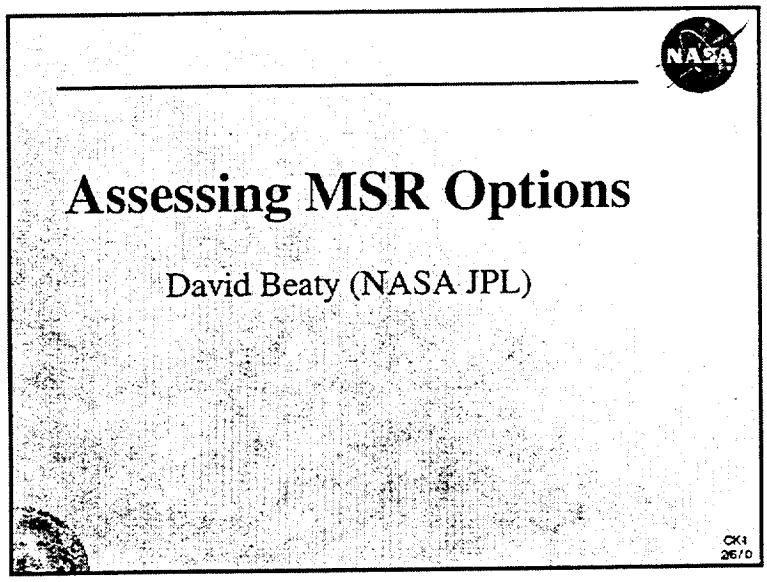
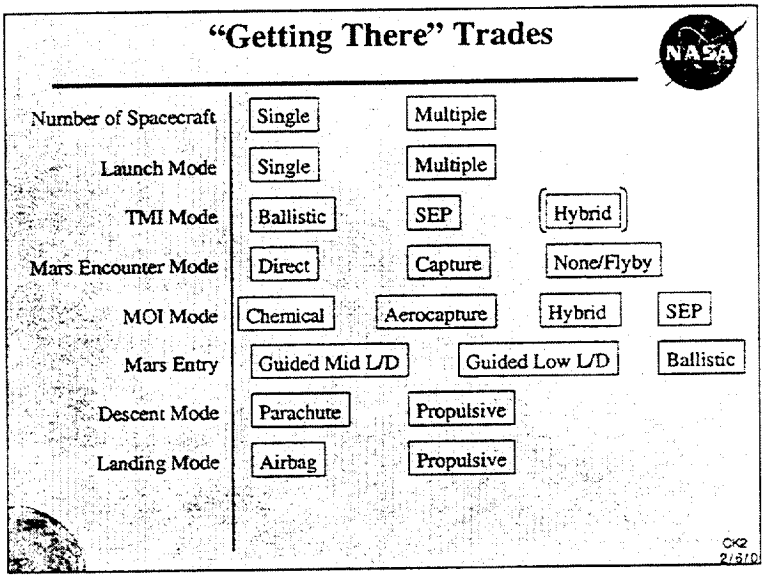

Scientific Reconnaissance as a Pathfinder to Where to Investigate
Today: MGS Map of Mars (MOC + MOLA)
MGS / MOC
MER Site
Carbonates
Targeting Trajectory
MGS: 100's of promising sites
Odyssey: Evaluate MGS sites, identify new ones
MER: Validate MGS/Odyssey data, explore 2 promising sites
MRO: For each MGS/Odyssey site, investigate its promise
"Smart": Mobile Laboratory: Go to the best site(s) to prospect for life
Odyssey / THEMIS
MRO
Descent imaging (3 x 6 km)
Insert as calibration back into global framework
"Smart" Mobile Laboratory Landing Site
Carbonates or clays predicted in layered sediment stack
Touchdown at a "hot spot"

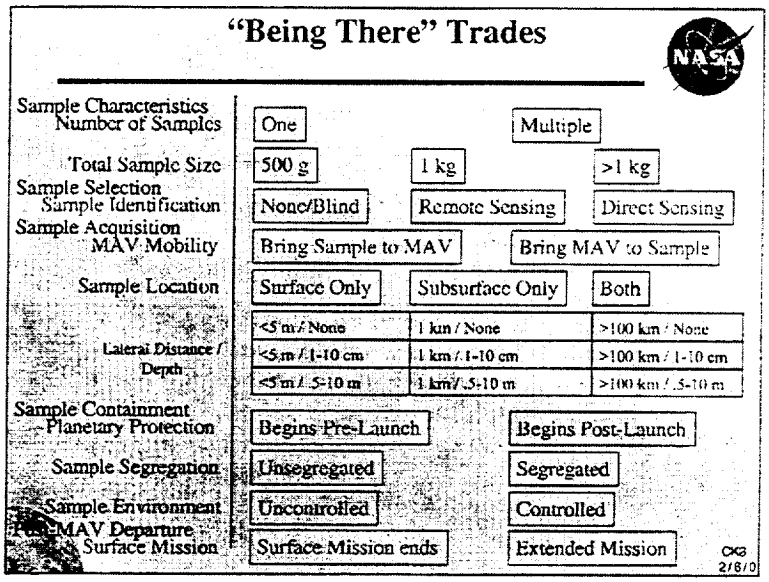

MARS EXPLORATION
New NASA Roles and Responsibilities
Headquarters Roles and Positions
• Mars Program Director (Hubbard) has full budget, requirements and program authority from formulation through development and into operations
• Mars Lead Program Scientist (Garvin) defines Mars science requirements for the program
Lead Center (JPL) Roles and Positions
• Mars Program Manager (Naderi) has full responsibility for program implementation (subject to HQ requirements), including studies, flight development and mission operations
• Space Science Flight Projects Director (Gavin) is responsible for project implementation from the definition phase through hardware delivery and launch

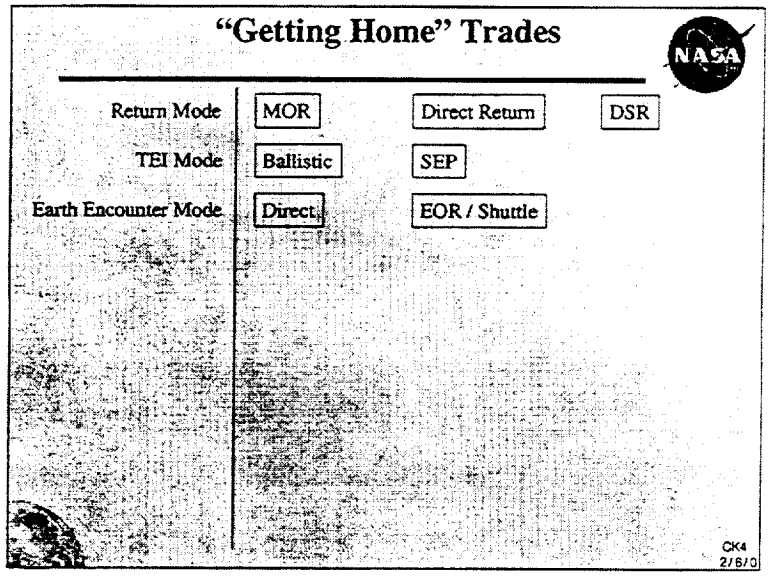
Mars Sample Return Mission Planning

David Beaty, NASA Jet Propulsion Laboratory



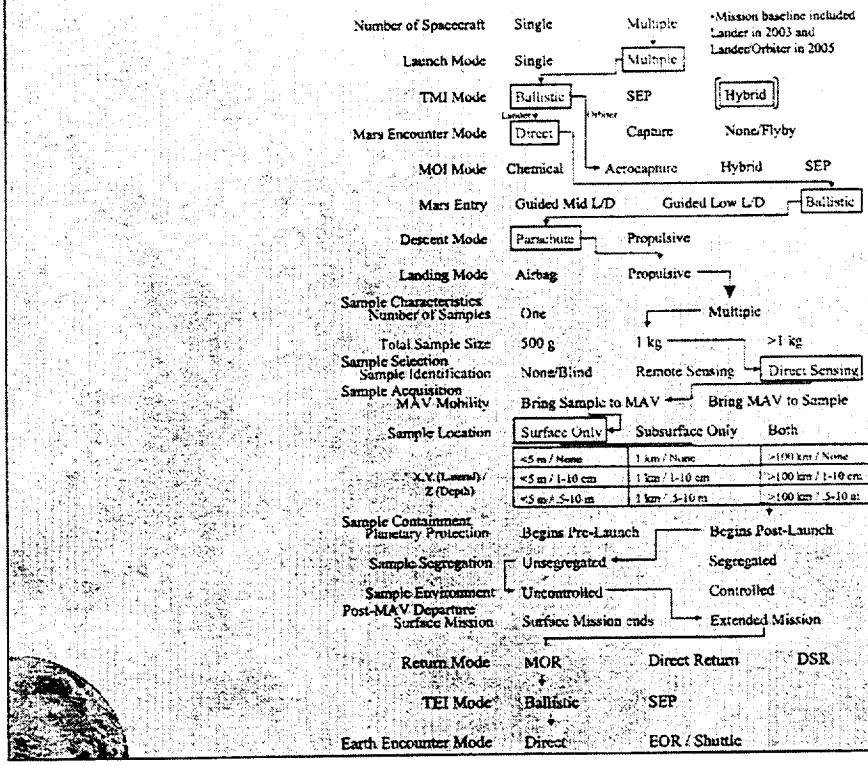



"Being There" Trades
Sample Characteristics
Number of Samples
One
Multiple
Total Sample Size
500 g
1 kg
>1 kg
Sample Selection
Sample Identification
None/Blind
Remote Sensing
Direct Sensing
Sample Acquisition
MAV Mobility
Bring Sample to MAV
Bring MAV to Sample
Sample Location
Surface Only
Subsurface Only
Both
Lateral Distance / Depth
<5 m / None
1 km / None
>100 km / None
<5 m / 1-10 cm
1 km / 1-10 cm
>100 km / 1-10 cm
<5 m / 5-10 m
1 km / 5-10 m
>100 km / 5-10 m
Sample Containment
Planetary Protection
Begins Pre-Launch
Begins Post-Launch
Sample Segregation
Unsegregated
Segregated
Sample Environment
Uncontrolled
Controlled
Post-MAV Departure & Surface Mission
Surface Mission ends
Extended Mission
CK3
2/6/0


"Getting Home" Trades
Return Mode
MOR
Direct Return
DSR
TEI Mode
Ballistic
SEP
Earth Encounter Mode
Direct
EOR / Shuttle
CK4
2/6/0

Full Trade Space Example

2003/2005 MSR Baseline



Many Quantitative Objective Functions Are Possible

- Maximize "expected mission value" return ... [covers ev difficult to carry out]
- Maximize science return divided by (risk times cost) ... [
- Maximize probability of mission success for "floor scienc [Kohlhastkes]
- Minimize program costs for floor science
- Minimize schedule risk for 2011 MSR
- Maximize introduction of new technology to benefit futu HEDS missions
- Minimize dependence on new technology, relying on pro
- Minimize launch mass to meet floor science
- Consider many different weighted blends of the above

Note: "floor science" is ... return 500 g of Martian soil and rock ... plus ...

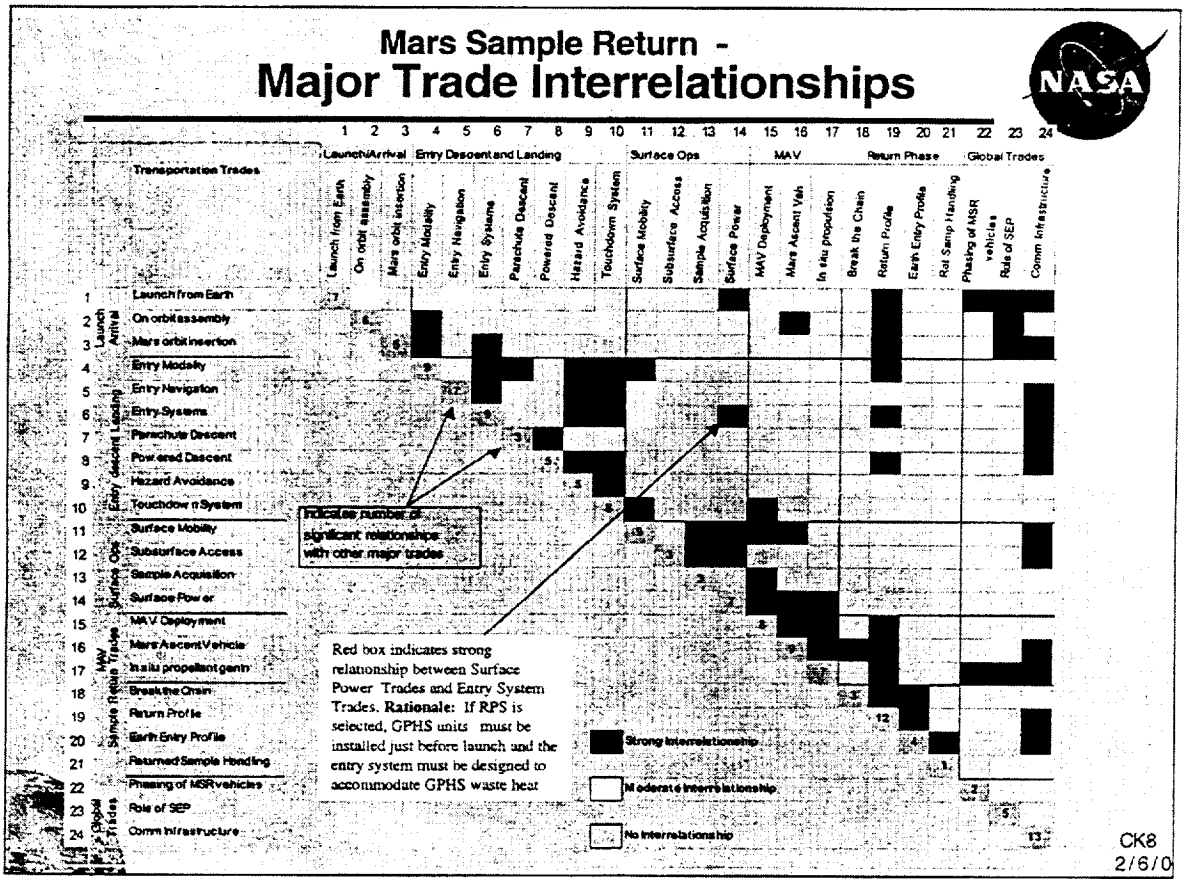
CK6
2/6/0

Mars Sample Return
Major Trade Interrelationships

- Domain of major trades is enormous – parsing v conventional trade tree approaches is very diffi
- Most major trades can be arranged in a chronol sequence by mission phase – from Earth depar Earth return
- This circumstance permits a useful examination interrelationships between trades using a matri representation
- This representation enables
  - Systematic identification of all major trade interrelationship
  - Recognition of those trades with the strongest cross coupling trades

CK7
2/6/0

Mars Sample Return -
Major Trade Interrelationships



CK8
2/6/0

Report of the LIFARS Workshop

Kenneth Nealson, NASA Jet Propulsion Laboratory
and David Blake, Ames Research Center

Does the sample contain evidence for extant life?				
Technique	Performance	Advantages	Limitations	Developments needed
Light Microscopy, optical broadband spectroscopy	0.2 µm spatial resolution	non-invasive	spatial res.	none
CT scan, XAFS imaging	5 µm spatial resolution	non-invasive, looks inside	spatial res.	higher spatial resolution, monochromator
XRD/XRF	200 µm spatial resolution	non-invasive	minerals, elements only	higher spatial resolution
IR Spectroscopy/Raman	1 µm spatial resolution	non-invasive	signal/noise	ultrastable sources, low noise detectors
UV Fluorescence/Raman	1 µm spatial resolution	non-invasive	signal/noise	Low-noise detectors
Electron Microscopy (ESEM, SEM, EDX)	1-10 nm, 0.2 KeV, 1% rel. abundance	high resolution morphology, comp.	sample prep.	contamination-free microscopes
Electron Microscopy (TEM, SAED, EELS)	1 nm, .5 eV, 1-10% rel. abundance	structure, redox. State, mineralogy	sample prep.	better sample prep.
Vertical Scanning Interferometry	0.2 nm in z, 100 nm in x,y	real time, in situ	spatial res.	higher resolution, in situ
Laser desorption / Laser ionization TOF, MALDI, ESI	10^{-20} moles	intact biomolecules	molecular weights only	better lasers, sample prep.
CHONS isotope analysis	pmole-nmole	integrates signs of life	sample prep, not definitve	chromatography, ion source
Chip chromatography-micro-array antibody binding	single molecule	highly specific, small sample mass	specific sensors needed	sensors / aptomers, detectors & array development
Chromatography for chirality, capillary zone electrophoresis CZE)	pmol	sensitive	sample prep, non-specific	derivatization
Metabolic analysis	test-specific	direct method	sensitivity	lots of work
Multi-photon detection	10^{-21} moles	sensitive, specific	radioactive contamination	labeled probes, detectors
Microcalorimetry	ergs	sensitivity to reactions	sample prep	sample prep
NMR Spectroscopy	10 µm spatial resolution	imaging, looks inside	Fe content limits analysis	sensitivity, probe development
GC/MS	mass res. 1:60,000 10^{-15}-10^{-18} mol	lipid biomarkers	optimized for small molecules	ionization techniques

Does the sample contain evidence for past life?				
Technique	Performance	Advantages	Limitations	Developments needed
Light microscopy	0.2 µm spatial resolution	non-invasive	spatial res.	none
CT scan, XAFS imaging	5 µm spatial resolution	non-invasive, looks inside	spatial res.	higher spatial resolution, monochromator
XRD/XRF	200 µm resolution	non-invasive	minerals, elements only	higher spatial resolution
IR spectroscopy/Raman	1 µm spatial resolution	non-invasive	signal/noise	ultrastable sources, low noise detectors
UV fluorescence/Raman	1 µm spatial resolution	non-invasive	signal/noise	low noise detectors
Electron Microscopy (ESEM, SEM, EDX)	1-10 nm, 0.2 KeV, 1% rel. abundance	high resolution morphology, comp.	sample prep	contamination-free microscopes
Electron Microscopy (TEM, SAED, EELS, EDX)	1 nm res., 0.5 eV, 1-10% rel. abundance	structure, redox state, mineralogy	sample prep	better sample prep.
Vertical Scanning Interferometry	0.2 nm in z, 100 nm in x,y	real time, in situ	wavelength (diffraction)	higher resolution, in situ
Laser desorption / Laser ionization TOF, MALDI, ESI	10^{-12} moles	high mol. wt. organic molecules	molecular weights only	sample prep
CHONS isotope analysis	pmol-nmol	integrates signs of life	sample prep, not definitve	understand diagenesis, chromatography, ion source
Chip chromatography-µ array, antibody binding	100 molecules minimum	highly specific, small sample mass	preservation, diagenesis	understand diagenesis
Chromatography for chirality, Capillary zone electrophoresis	pmol	sensitive	sample prep, non-specific	understand racemization after death
NMR Spectroscopy	10 µm	imaging, looks inside; chem. inventory	quenching; specific elements	probe development
Fluid inclusion/microthermometry	1 µm spatial resolution	determines environment	not specific, sample prep.	integrate with other spectroscopic techniques
Ion microprobe	10 nm	small sample, environmental context	sample prep, poor dynamic spatial range	higher efficiency
Whole rock isotopic analysis		stable signal	not specific	control studies
GC/MS	mass res. 1:60,000, 10^{-15}-10^{-18} mol	lipid biomarkers	optimized for small molecules	ionization techniques

Does the sample contain evidence for prebiotic organic chemistry?				
Technique	Performance	Advantages	Limitations	Developments needed
XRD/XRF	10 m	No sample prep	No organic	Probe size, resolution
CT scan/XAFS imaging	5 m	Non-invasive, looks inside	Spatial res.	Higher spatial resolution, monochromator
Whole rock isotopic analysis		Stable signal	Not specific	Control studies
Fluid inclusion/microthermometry	1 m	Determines environment	Not specific, sample prep	Integrate with other spectroscopic techniques
IR spectroscopy/Raman	1 m	Non-invasive	SNR	Ultrastable sources, low noise detectors
UV fluorescence/Raman	1 m	Non-invasive	SNR	Low noise detectors
Ion microprobe	10 nm	Small sample, environmental context	Sample prep, poor dynamic spatial range	Higher efficiency
TEM/EELS/SAED	1 nm, 0.05 eV, 10-15% rel. abundance	Redox state, mineralogy	Sample prep	EELS detector
Electron beam/EDX	1-100 nm, 0.2 keV, 10% rel. abundance	Chemistry	wavelength (diffraction)	Higher resolution, in situ
Chip chromatography-m array	Single molecule	Highly specific, small sample mass	Specific sensors needed	Detectors, array development
Laser desorption TOF	10^{-15} moles	Intact biomolecules	Molecular weights only	Better lasers, sample prep
CHONS isotope analysis	pmol-nmol	Integrates signs of life	Sample prep, not definitve	Chromatography, ion source
Chromatography for chirality	pmol	Sensitive	Sample prep, non-specific	Derivatization
NMR Spectroscopy	10 m	Imaging, looks inside; chem. inventory	Quenching; specific elements	Probe development
GC/MS	mass res. 1:60,000, 10^{-15}-10^{-18} mol	Lipid biomarkers	Optimized for small molecules	Ionization techniques
Mossbauer spectroscopy	Bulk	Fe valence	only Fe	None
AFM/CFM	0.1-1 nm	Chemistry	Sample prep	Larger field of view

Does the sample contain evidence of the environment for prebiotic chemistry?				
Technique	Performance	Advantages	Limitations	Developments needed
CT scan/XAFS imaging	5 m	Non-invasive, looks inside	Spatial res.	Higher spatial resolution, monochromator
IR spectroscopy/Raman	1 m	Non-invasive	SNR	Ultrastable sources, low noise detectors
UV fluorescence/Raman	1 m	Non-invasive	SNR	Low noise detectors
TEM/EELS/SAED	1 nm, 0.05 eV, 10-15% rel. abundance	Redox state, mineralogy	Sample prep	EELS detector
Electron beam/EDX	1-100 nm, 0.2 keV, 10% rel. abundance	Chemistry	wavelength (diffraction)	Higher resolution, in situ
Chip chromatography-m array	100-1000 molecules	Small sample mass	Molecules not yet characterized	Probes for non-living organic matter needed
Laser desorption TOF	10^{-12} moles	High molecular weightbiomolecules	Molecular weights only	Better lasers, sample prep
CHONS isotope analysis	nmol	Distinguish kinetics vs. equilibrium processes	Sample prep, not definitve	Control expts. Needed, chromatography, ion source
Chromatography for chirality	pmol	Sensitive	Sample prep, non-specific	Extend derivatization for other molecules
NMR Spectroscopy	10 m	Imaging, looks inside; chem. Inventory	Quenching; specific elements	Probe development
GC/MS	mass res. 1:60,000, 10^{-15}-10^{-18} mol	Momomeric structural information	Optimized for small molecules, derivatization needed	Sample introduction, ionization techniques

Report of the NAS Life Detection Workshop

John Baross, University of Washington

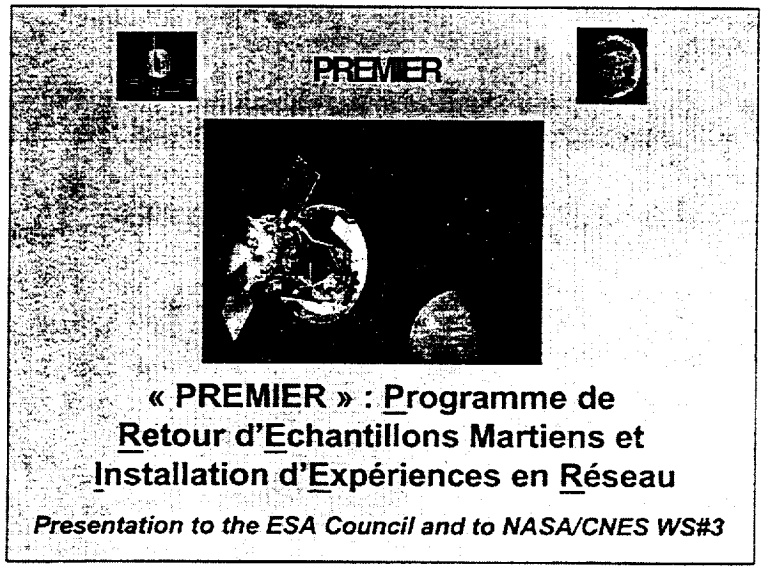
As part of the charge to the recent NRC study of "The Quarantine and Certification of Martian Samples," the Committee on Planetary and Lunar Exploration (COMPLEX), was asked to evaluate what criteria must be satisfied before martian samples can be released from the SRF. The Study Committee was chaired by John Wood (Harvard University).

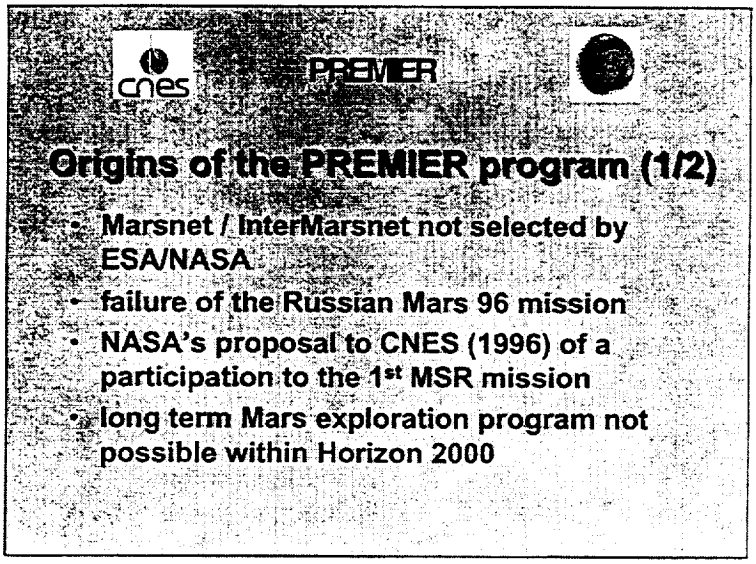
John Baross summarized the Committees' progress to date. Since the Study Committee had just completed their deliberations at the time of Workshop 3 therefore no visual materials were used to describe this work.

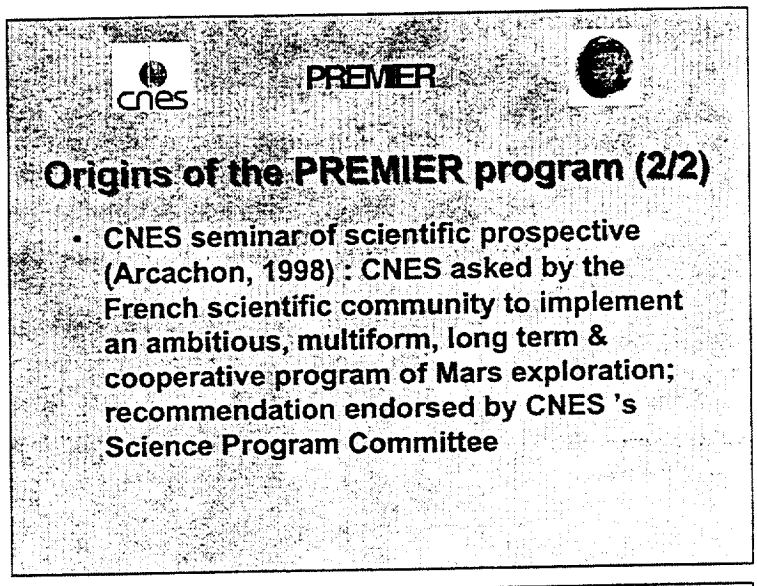
The final report of the COMPLEX Committee is *in press* at the time of the preparation of this report.

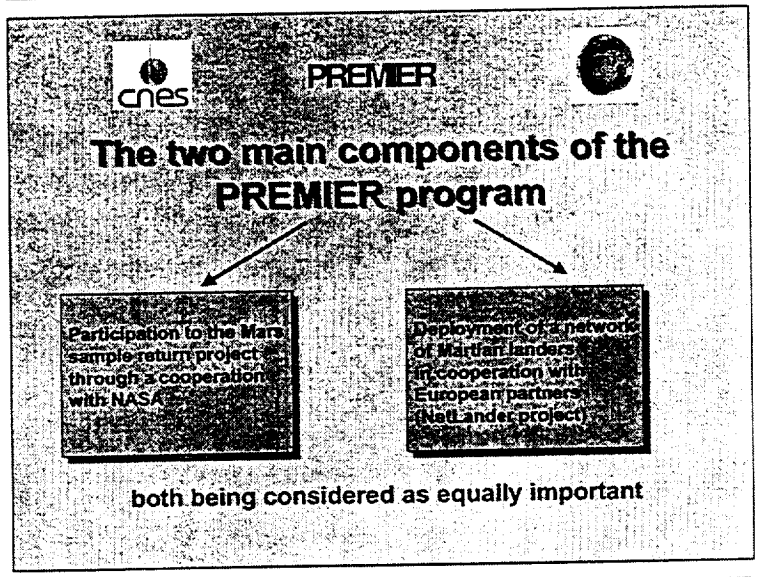
French Planning for Mars Missions and Sample Return: *PREMIER*

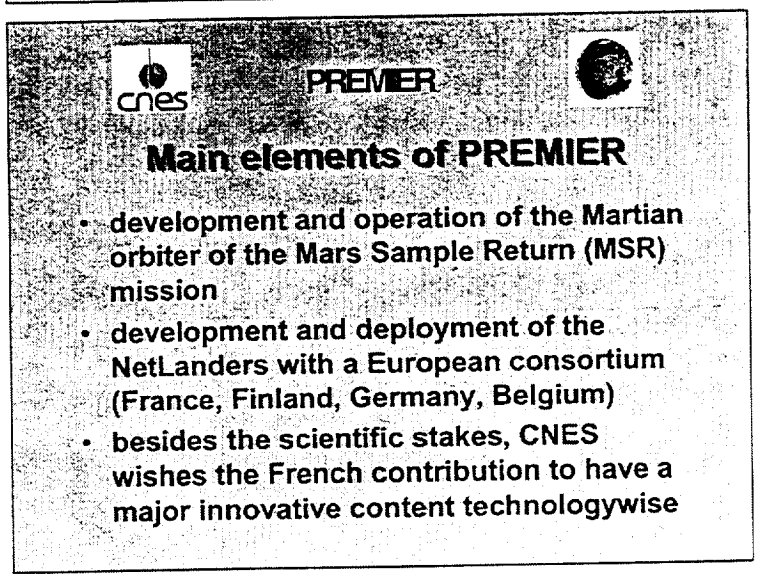
Michel Viso, Centré National d'Etudes Spatiale






cnes
PREMIER
Origins of the PREMIER program (2/2)
• CNES seminar of scientific prospective (Arcachon, 1998) : CNES asked by the French scientific community to implement an ambitious, multiform, long term & cooperative program of Mars exploration; recommendation endorsed by CNES 's Science Program Committee


cnes
PREMIER
The two main components of the PREMIER program
Participation to the Mars sample-return project through a cooperation with NASA
Deployment of a network of Martian landers in cooperation with European partners (NetLander project)
both being considered as equally important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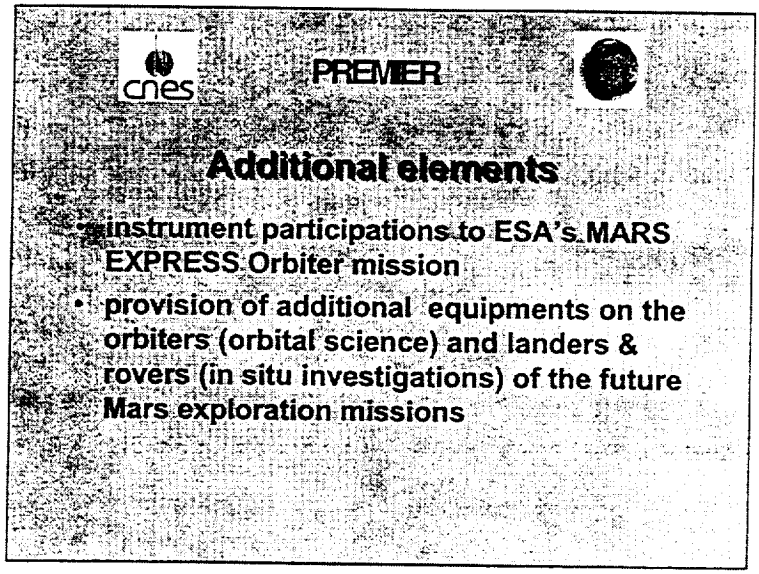
PREMIER
Main elements of PREMIER
• development and operation of the Martian orbiter of the Mars Sample Return (MSR) mission
• development and deployment of the NetLanders with a European consortium (France, Finland, Germany, Belgium)
• besides the scientific stakes, CNES wishes the French contribution to have a major innovative content technologywise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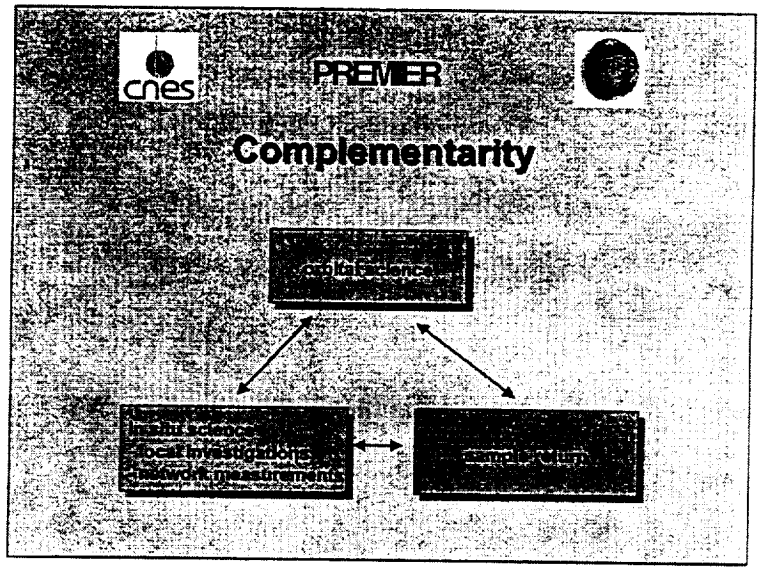
PREMIER
Additional elements
• instrument participations to ESA's MARS EXPRESS Orbiter mission
• provision of additional equipments on the orbiters (orbital science) and landers & rovers (in situ investigations) of the future Mars exploration missions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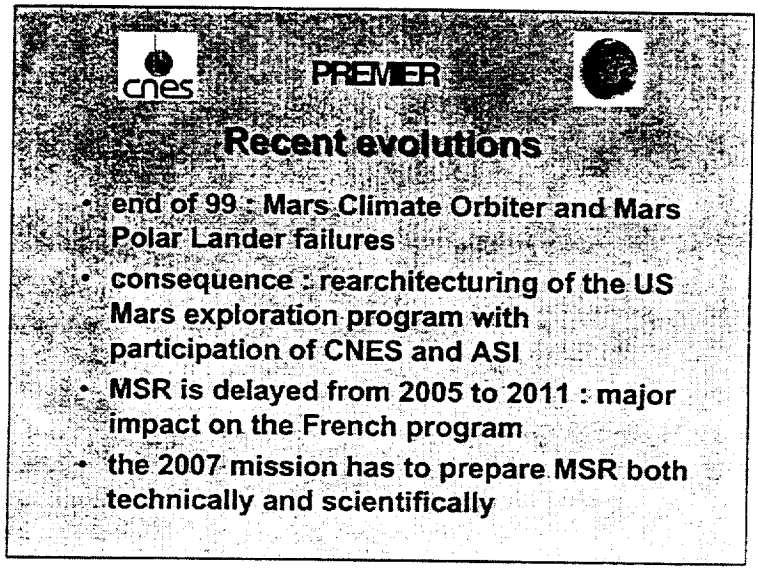
PREMIER
Complementarity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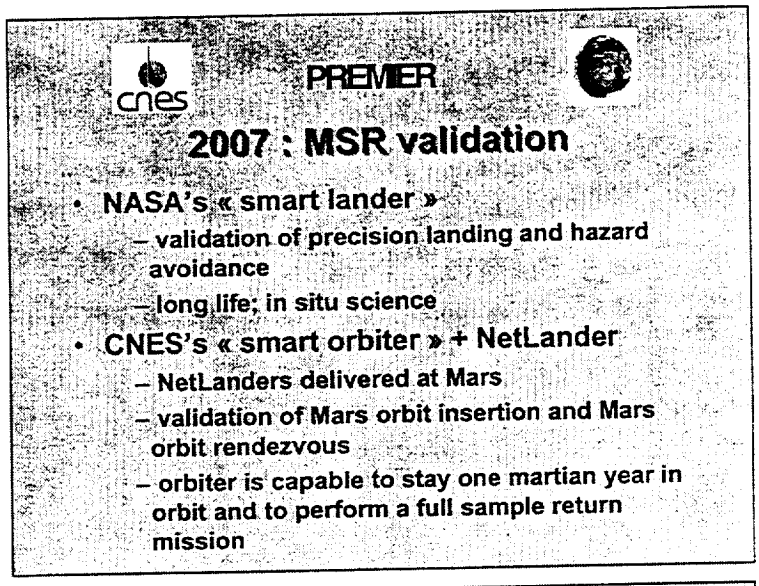
PREMIER
Recent evolutions
• end of 99 - Mars Climate Orbiter and Mars Polar Lander failures
• consequence : rearchitecturing of the US Mars exploration program with participation of CNES and ASI
• MSR is delayed from 2005 to 2011 : major impact on the French program
• the 2007 mission has to prepare MSR both technically and scientifically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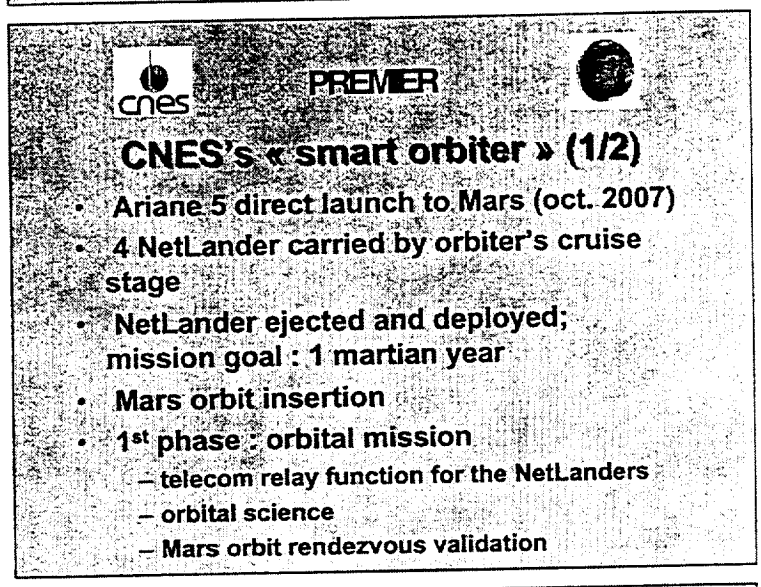
PREMIER
2007 : MSR validation
• NASA's « smart lander »
– validation of precision landing and hazard avoidance
– long life; in situ science
• CNES's « smart orbiter » + NetLander
– NetLanders delivered at Mars
– validation of Mars orbit insertion and Mars orbit rendezvous
– orbiter is capable to stay one martian year in orbit and to perform a full sample return mission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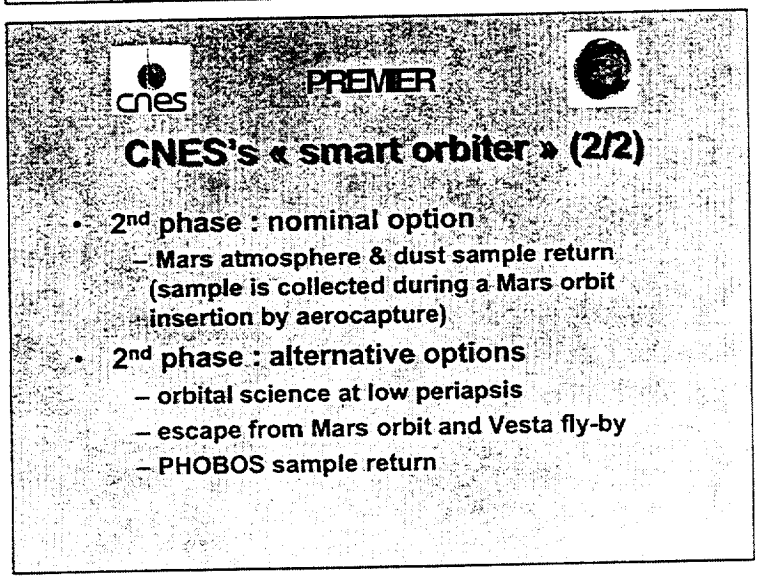
PREMIER
CNES's « smart orbiter » (1/2)
• Ariane 5 direct launch to Mars (oct. 2007)
• 4 NetLander carried by orbiter's cruise stage
• NetLander ejected and deployed; mission goal : 1 martian year
• Mars orbit insertion
• 1st phase : orbital mission
– telecom relay function for the NetLanders
– orbital science
– Mars orbit rendezvous validation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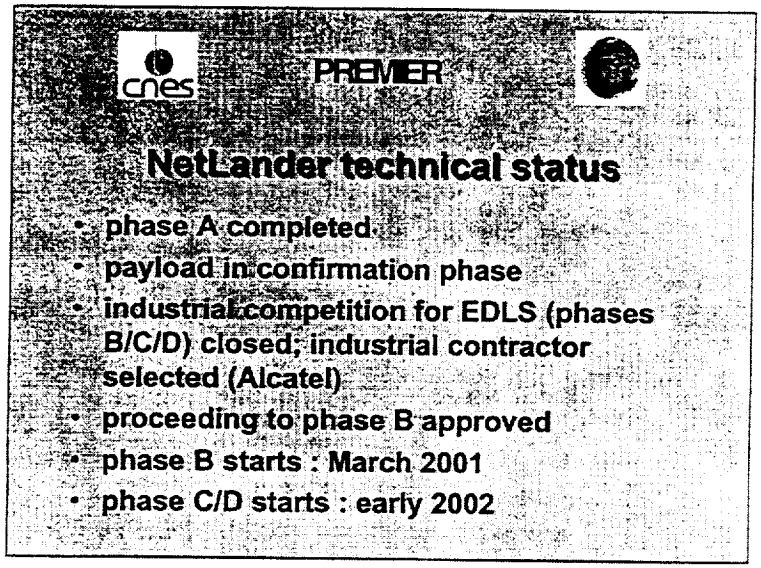
PREMIER
CNES's « smart orbiter » (2/2)
• 2nd phase : nominal option
– Mars atmosphere & dust sample return (sample is collected during a Mars orbit insertion by aerocapture)
• 2nd phase : alternative options
– orbital science at low periapsis
– escape from Mars orbit and Vesta fly-by
– PHOBOS sample return

cnes
PREMIER
2011 : 1st Mars sample return mission
• NASA « smart lander » + sample collection device + Mars Ascent Vehicle
• CNES « smart orbiter » + full Orbiting Sample Canister Rendezvous device « OSCAR » (options : Earth Entry Vehicle or Shuttle rendezvous)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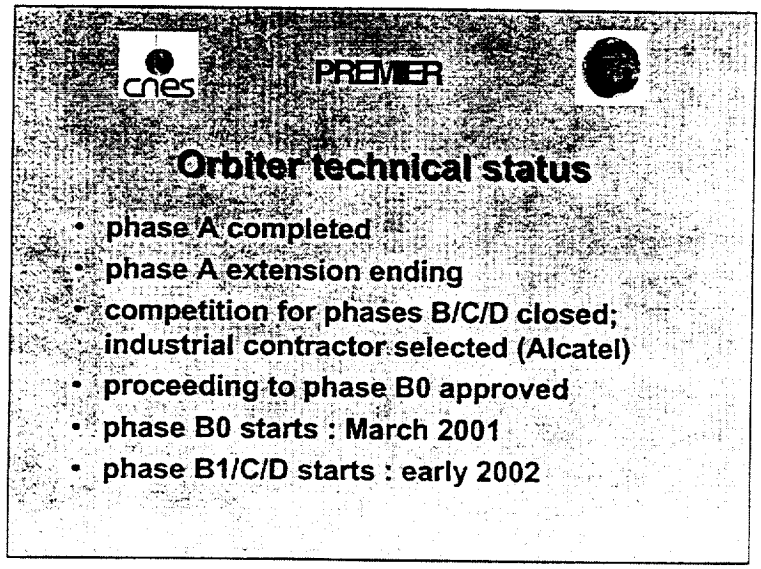
PREMIER
NetLander technical status
• phase A completed
• payload in confirmation phase
• industrial competition for EDLS (phases B/C/D) closed; industrial contractor selected (Alcatel)
• proceeding to phase B approved
• phase B starts : March 2001
• phase C/D starts : early 2002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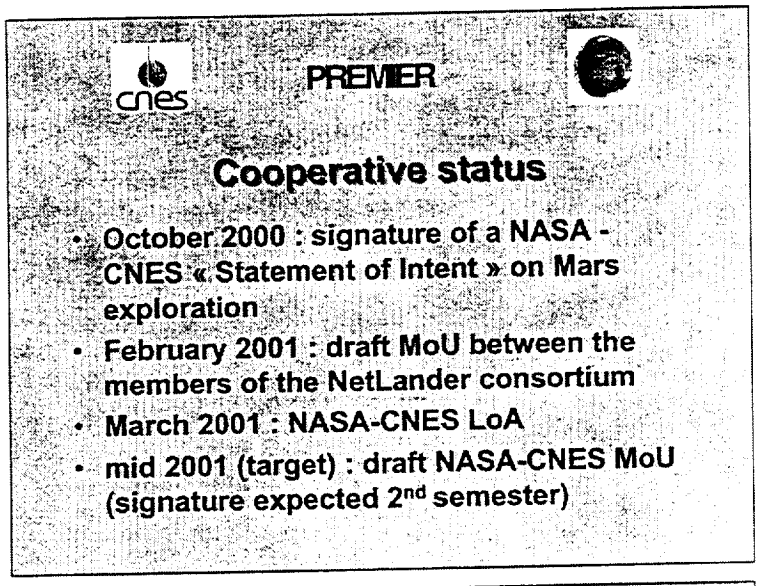
PREMIER
Orbiter technical status
• phase A completed
• phase A extension ending
• competition for phases B/C/D closed; industrial contractor selected (Alcatel)
• proceeding to phase B0 approved
• phase B0 starts : March 2001
• phase B1/C/D starts : early 2002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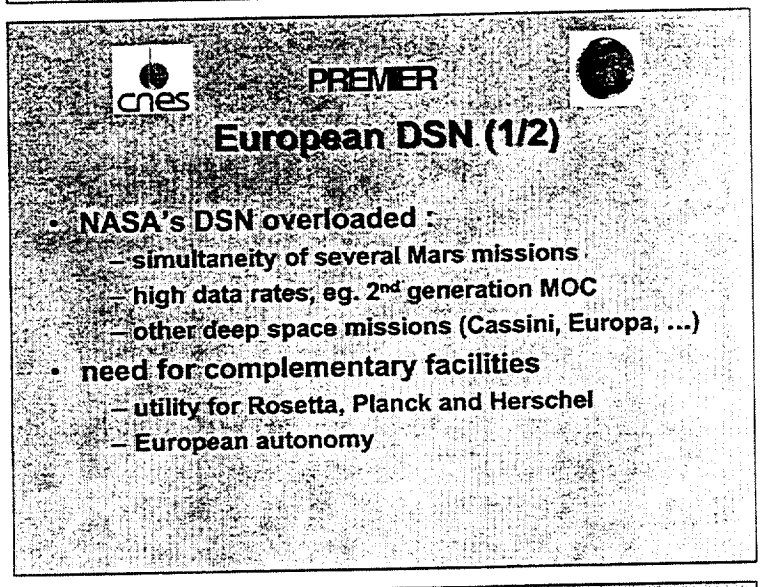
PREMIER
Cooperative status
• October 2000 : signature of a NASA - CNES « Statement of Intent » on Mars exploration
• February 2001 : draft MoU between the members of the NetLander consortium
• March 2001 : NASA-CNES LoA
• mid 2001 (target) : draft NASA-CNES MoU (signature expected 2nd semester)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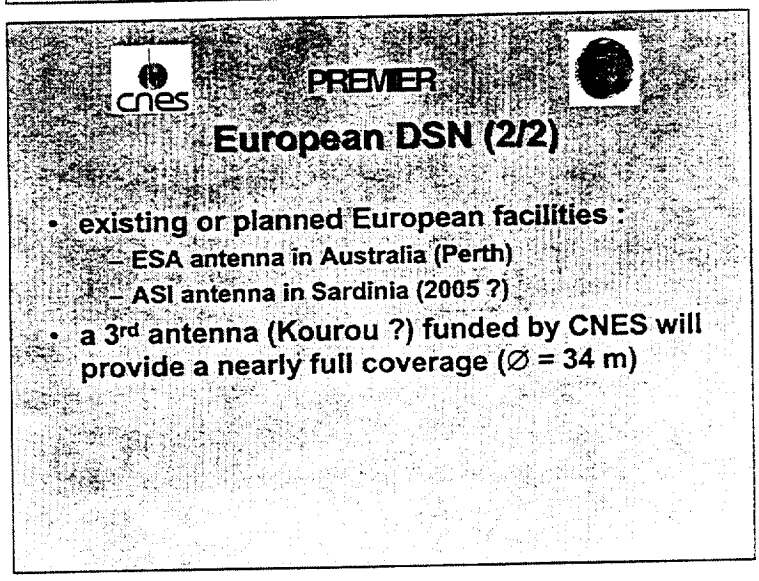
PREMIER
European DSN (1/2)
• NASA's DSN overloaded :
– simultaneity of several Mars missions
– high data rates, eg. 2nd generation MOC
– other deep space missions (Cassini, Europa, ...)
• need for complementary facilities
– utility for Rosetta, Planck and Herschel
– European autonomy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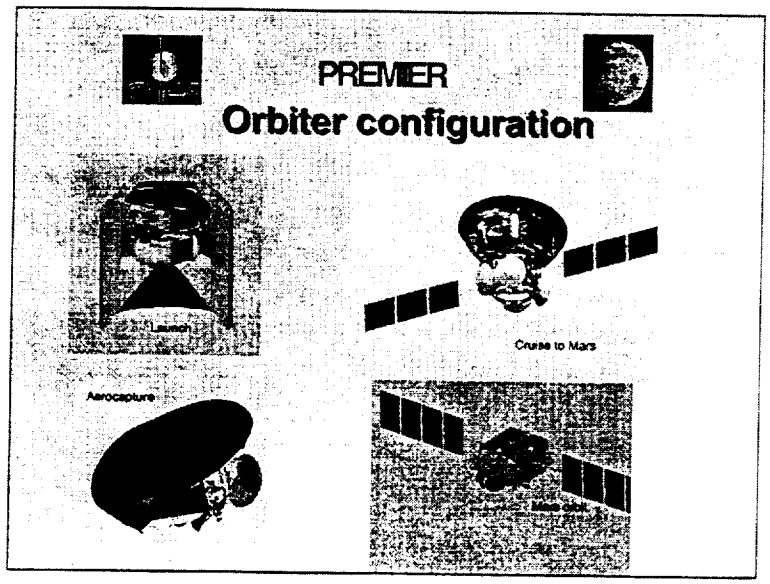
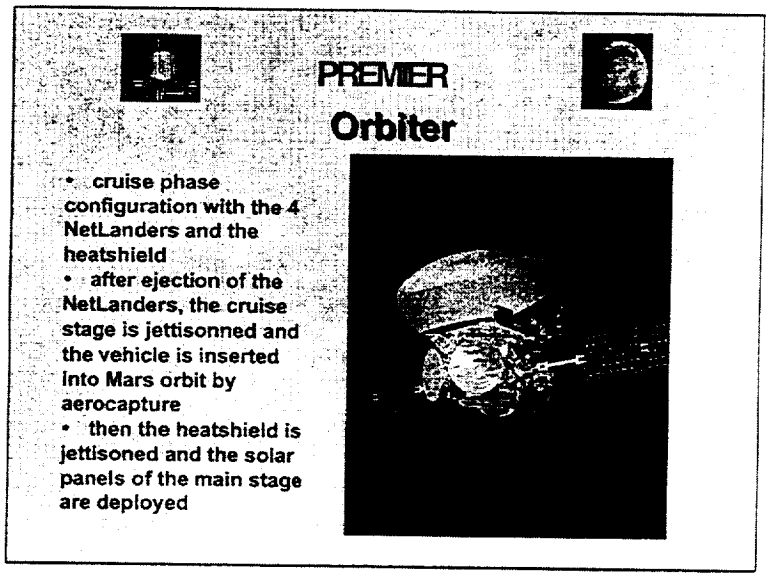
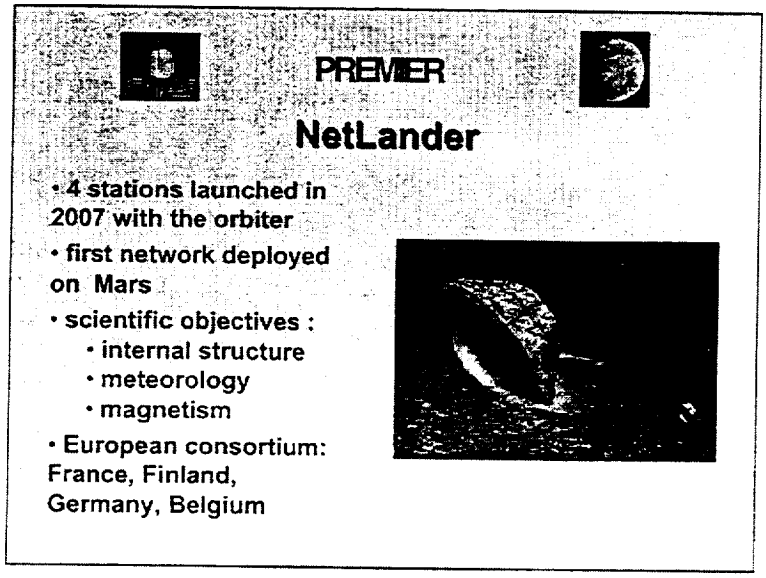
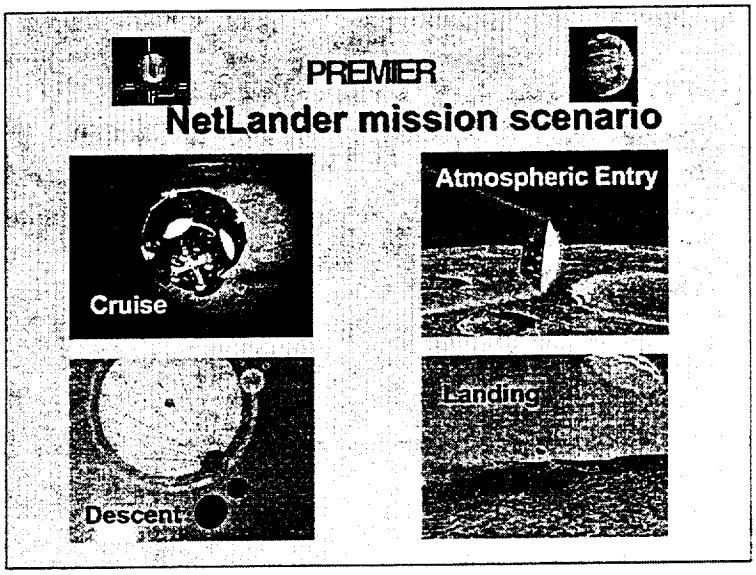
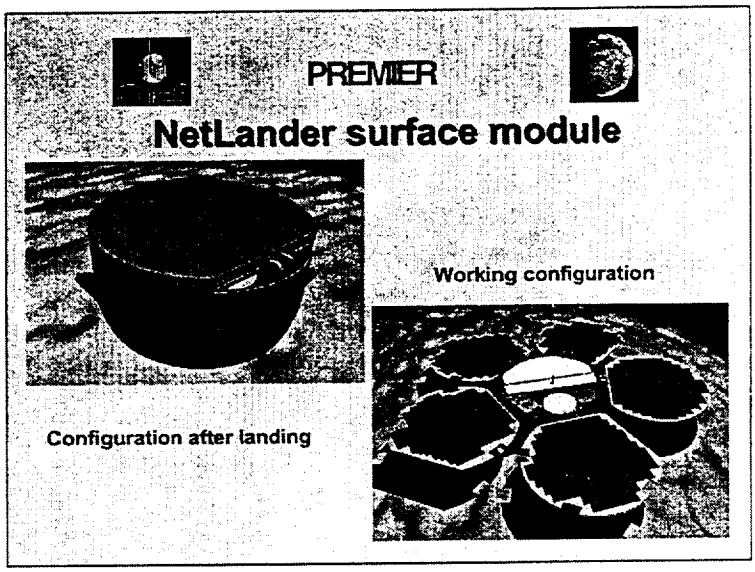
PREMIER
European DSN (2/2)
• existing or planned European facilities :
– ESA antenna in Australia (Perth)
– ASI antenna in Sardinia (2005 ?)
• a 3rd antenna (Kourou ?) funded by CNES will provide a nearly full coverage (∅ = 34 m)

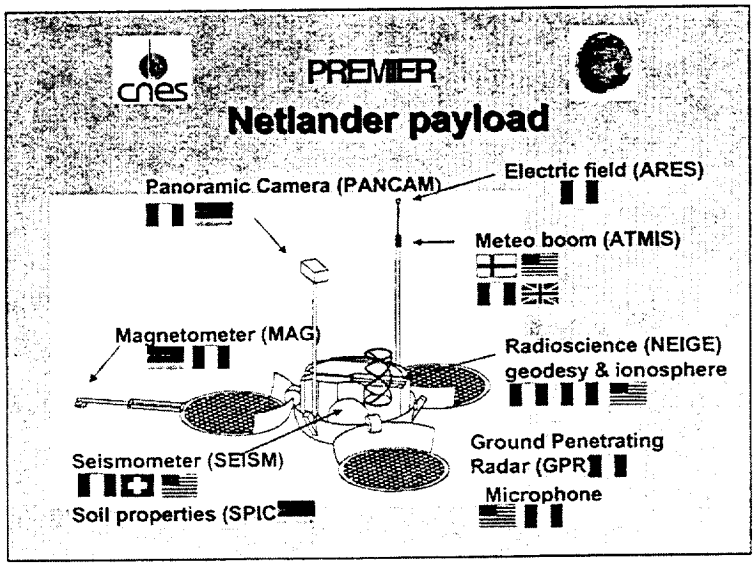

PREMIER
Orbiter configuration
Launch
Cruise to Mars
Aerocapture


PREMIER
Orbiter
• cruise phase configuration with the 4 NetLanders and the heatshield
• after ejection of the NetLanders, the cruise stage is jettisonned and the vehicle is inserted into Mars orbit by aerocapture
• then the heatshield is jettisoned and the solar panels of the main stage are deployed


PREMIER
NetLander
• 4 stations launched in 2007 with the orbiter
• first network deployed on Mars
• scientific objectives :
• internal structure
• meteorology
• magnetism
• European consortium: France, Finland, Germany, Belgium


PREMIER
NetLander mission scenario
Cruise
Atmospheric Entry
Descent
Landing


PREMIER
NetLander surface module
Working configuration
Configuration after landing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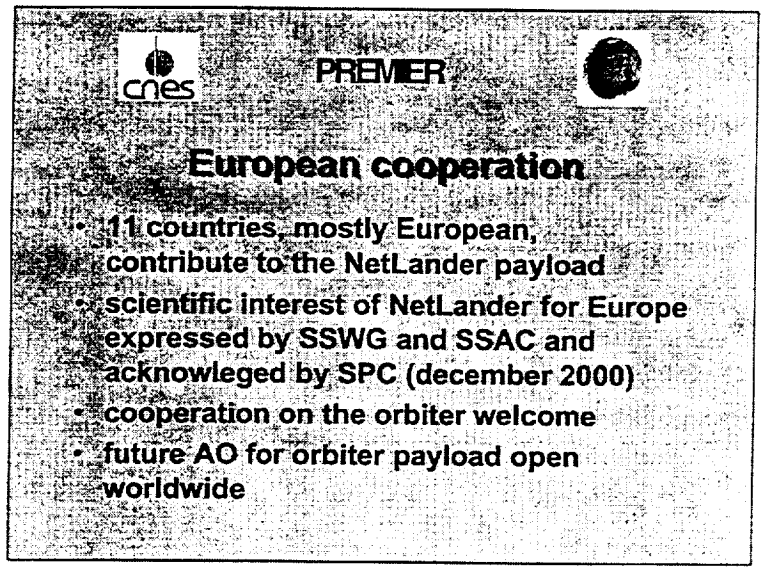
PREMIER
Netlander payload
Panoramic Camera (PANCAM)
Electric field (ARES)
Meteo boom (ATMIS)
Magnetometer (MAG)
Radioscience (NEIGE)
geodesy & ionosphere
Ground Penetrating
Radar (GPR)
Seismometer (SEISM)
Microphone
Soil properties (SPIC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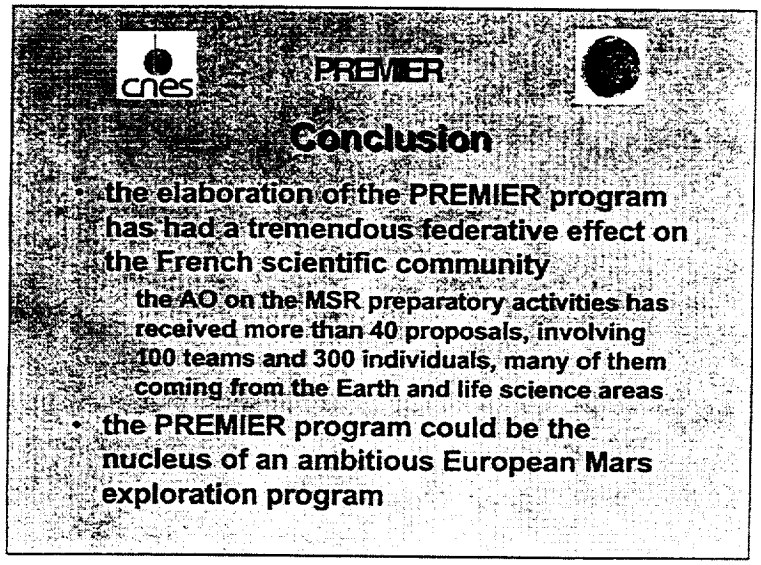
PREMIER
European cooperation
• 11 countries, mostly European, contribute to the NetLander payload
• scientific interest of NetLander for Europe expressed by SSWG and SSAC and acknowleged by SPC (december 2000)
• cooperation on the orbiter welcome
• future AO for orbiter payload open worldwide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PREMIER
Conclusion
• the elaboration of the PREMIER program has had a tremendous federative effect on the French scientific community
the AO on the MSR preparatory activities has received more than 40 proposals, involving 100 teams and 300 individuals, many of them coming from the Earth and life science areas
• the PREMIER program could be the nucleus of an ambitious European Mars exploration program

Summary of MSHP Workshops 1 & 2

Margaret S. Race, SETI Institute and Gregory T.A. Kovacs, Stanford University

Mars Sample Handling Protocol (MSHP) Workshop Series Interim Report – Workshop #1

March 20-22, 2000 - Bethesda, MD

M.S. Race and J.D. Rummel, Editors

Workshop Series Objectives:

1)Develop comprehensive protocols to assess that returned materials do not contain biological hazards

2)Safeguard the purity of the samples from possible Earth contaminants.

Extend & Refine Recommendations from Other Workshops:

1) Mars Sample Quarantine Protocol Workshop, NASA Ames (1997)
2) Post-Apollo updating of *Biocontainment, Life Detection, and Biohazard Testing*
3) MSHRP - Mars Sample Handling Requirements Panel (1999)
   - *Sample collection and transport back to Earth*
   - *Certification of the sample as non-hazardous*
   - *Sample receiving, curation, and distribution*

Intended Use:

- Assist NASA's Planetary Protection Officer and senior administrators in *preparing for* Mars ***sample return facilities, technology, and operations***;
- Serve as a ***briefing document*** for advisory groups, regulatory agencies and other entities who will ultimately establish and review sample return handling policy, requirements and implementation, and
- ***Provide recommendations in a form suitable for use as input for possible future announcements of opportunity*** soliciting proposals for Mars sample handling.

General format:

1) *Tutorials* – To give participants a common basis in the technical areas necessary to achieve the objectives of the workshop. Topics included: Mission Architecture and Design; Planetary Protection; Science; Review of Apollo Quarantine and Relevant Reports.
2) *Starting Assumptions* – 12 Assumptions related to Sample Collection and Transport Logistics, Nature of Sample, Containment and Non-Contamination Needs, SRF Capabilities and Expectations (including sterilization TBD)
3) *Sub-Group Breakout Sessions* – with Assigned Tasks
4) *Plenary Sessions* – for Presentation and Discussion
5) *Written reports* by Sub-Group Chairs

Sub-groups

Topics 1-3 (~ 2 hours each):

1) Preliminary Sample Characterization Requirements
2) Representative Sub-Samples; Nature of Sample
3) Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria

Topics 4-6 (a full day each):

4) Physical/Chemical Analyses – Methods, Sample State, Containment Controls.
5) Candidate Life Detection Tests – Qualifiers, Contraindications, Controls, Characterization
6) Candidate Biohazard Tests – Qualifiers, Contraindications, Controls, Characterization

Sub-group Summaries intended to:

- Summarize relevant background information
- Provide an overview of deliberations to date
- Help frame issues that need further attention or resolution in upcoming workshops

Disclaimers:

- Findings preliminary (*NOT* recommendations...)
- May be inconsistent between subgroups
- Not represent a consensus of all workshop participants,
- May be inconsistent with final report & recommendations of series

Sub-group 1: Preliminary Sample Characterization Requirements

William Fishbein (Chair) and Marie-Christine Maurel(Co-Chair)

Charter:

4) Identify Information about the samples to enable effective life-detection and/or biohazard testing.
5) Focus on characteristics determinative in understanding results of in vitro and in vivo testing (site of collection; preservation conditions; physical/chem. characteristics)

Specific data and information to be collected:

1) *In situ* collection info
2) Sample conditions in transit to Earth
3) Physical characteristics of each specimen
4) Microscopic examination and cross sections
5) Elemental abundances
6) Mineralogical characterization
7) Non-destructive evaluation of cracks and defects in pristine rock samples
8) Surface reactivity and chemistry
9) Evaluation of total and organic carbon.
10) Discussions and R&D on sterilization of sub-samples prior to distribution (methods and implementation).

Combined Sub-groups 2 and 4 (one report)

Subgroup 2: Representative Sub-samples: Nature of Sample
Subgroup 4: Physical/Chemical Analyses, Methods, Sample State, Containment Controls

- Participants largely overlapped because of their expertise;
- Discussions complemented each other (nature & characterization of samples)
- Single report

Sub-group 2: Representative Sub-Samples; Nature of Sample
Glenn MacPherson (Chair) and Jean-Pierre Bibring (Co-Chair)

Original Task:
Specify and Recommend:

- Preliminary characterization data for partitioning samples into representative sub-sample allocations for testing,
- Process whereby samples can be sub-sampled effectively.
- Information that should be obtained within containment
  -To support sample characterization for later scientific analysis,
  -Time-critical measurement requirements,
  -Understand long-term preservation of the samples in curation

Sub-group 4: Physical/Chemical Analyses: Methods, Sample State, Containment, Controls
Donald Bogard (Chair) and Bernard Marty (Co-Chair)

Original Task:

- Address desired methods for physical & chemical analyses to meet requirements of sample-analysis protocol, curation, and storage
- Methods assessed for ability to
  - obtain information with minimum destruction of sample
  - be performed inside containment or on sterilized samples outside

Revised (Combined) Sub-group Charter 2 & 4:
"Establish a protocol for documenting, subdividing, and characterizing the samples; specifying the nature and sequence of physical, chemical and mineralogic tests necessary to support the tasks of life detection, biohazard analysis and preliminary examination for the benefit of the scientific user community."

Operating Principles:
In devising the sequence and nature of tests, sample flow, and examination of the samples, the following principles must underlie all activities within the receiving facility:

- Tests and characterization activities to use the absolute minimum amount of sample to carry out the test.
- All handling, tests and characterization activities do the least harm to samples (non-destructive, non-invasive tests preferable).
- Processing and storage cold ; Also, a non-harmful environment (dry?) filled with a non-contaminating gas. (TBD)
- Geochemical & mineralogic analyses kept to the minimum required to support biohazard assessment, life detection, and characterization for future sample allocations.

Sub-groups 2 & 4: Proposed Procedural Flow Chart

1) **Sample Removal and Basic Documentation:**
   > Extract and filter the gas
   > Open the sample container,
   > Remove the sample
   > Record basic physical, photographic & curatorial info

2) **Preliminary Characterization**
   - Select representative samples (visual & gross geol/mineral exam)
   - Non-destructive, non-invasive methods to characterize materials
   - (Visual Optics, IR & UV Spectroscopy, Qualitative X-ray fluorescence)
   - Fraction selected for testing (remainder stored)

3) **Splitting**
   - Separate sample types by size or other criteria (for Protocol & Sci.)
   - Sample types distinguished (fines, pebbles, cores, complex rocks)

4) **Detailed Examination & Analysis** (Physical Chem. & Mineralogy)

Bulk Chemistry	Mineralogy
Total Carbon	Preliminary Organic Carbon Analyses
Total Water Assay	Petrography (X-ray Fluor. & X-ray Diffract.)

5) **Release from Containment/Dispensation:**
   Samples either released from containment or sterilized (depending on protocol tests results)

Sub-group 3: Sequence of Tests; Types of Testing Possible; Range of Results re: Release Criteria

Peter Jahrling (Chair) and David Sourdive (Co-Chair)

Address the end-to-end requirements of an effective sample-testing protocol (use strawman protocol as a point of departure)
- Sequence of testing
- Timing and availability of complementary test results
- Nature of the criteria for sample release for scientific analysis

Sub-group report focused on:
- Biohazard Assessment
- Biohazard Clearance
- Criteria for Release
- Clarify what questions should be answered by the sequence of tests performed for biohazard clearance.

Consistent with SSB recommendations, samples must be:
1) Clean - not contaminated with terrestrial organisms;
2) Contained - prevent contamination of the Earth's biosphere
3) Sterile - if any portion is removed from containment prior to completion of the rigorous analyses, it must be sterilized first .

Four constraints & starting assumptions:
1) Any genuine *martian life form must be kept under continued containment* whether it is hazardous or not;
2) *Toxicity should be tested*, but it is not a criterion for release;
3) *Life detection and biohazard testing partially overlap;* and
4) Biohazard testing explicitly should *emphasize analytic probes that can identify agents that might live, replicate or otherwise interact with terrestrial carbon-based systems.*

Four levels of questions and methodological approaches that should guide the biohazard testing

Table I: Sequence of Questions and Possible Strategies for Decisions about Release from Containment

Item	Question	Strategy
1	Is there anything that looks like a life-form? (sequential search for **structural indications of life forms**)	Beam synchrotron or other nondestructive high-resolution analytic probe, particularly one that would allow testing non-sterilized (yet still contained!) samples outside main facility.
2	Is there a **chemical signature of life**?	Mass spec. or other test systems (to be used in containment) that would identify asymmetry, special bonding, etc.
3	Is there any evidence of **self replication or replication in terrestrial living organism**?	Attempts to grow in culture or in cell culture, defined living organisms.
4	Is there **any adverse effect on workers or** the surrounding **environment**? (monitoring)	Medical surveillance; evaluation of living systems in proximity of the receiving facility

Four areas needing further attention:

1) Input from other government agencies w/ experience in biohazard testing
2) Deliberations on what cell and whole organism types should be used in biohazard assessment
3) Involvement of statistical experts in assessing the validity of sampling and testing plans
4) Research and consulting on development of microscale model systems for assessing potential impacts on ecosystems.

Subgroup 5: Candidate Life Detection Tests – Qualifiers, Contraindications, Controls, and Characterization

Mitchell Sogin (Chair) and Daniel Prieur (Co-Chair)

Task:

Preliminary identification of measurements and tests to look for evidence of life or life-related molecules.

- Identify Methods and Instrumentation if possible.
- Specify relationships of info from complementary life-detection tests

Approach:

Outlined a series of procedures that will minimally be required to assess for the presence of non-terrestrial life forms in returned martian samples (rocks, soils and fines).

Considered Three Possible Outcomes:

1) *No terrestrial-like life exists* as evidenced by the complete absence of carbon or complex carbon in the returned sample. (release)
2) *Clear and overwhelming evidence of living organisms* as demonstrated by self-replicating entities capable of evolving (continued containment & biological study).
3) *Most likely scenario: Complex carbon containing compounds are present, but without clear evidence of replicative properties.*

Flow Chart: Sequential processing of sample types

Variety of methods for different sample types:

Fluorescent activated flow cytometry	Filtration
Laser raman mass spectroscopy	LAL assays,
PCR sequencing,	Micro-scale culturing,
Broad Band Fluorescence	3-D tomography in a synchrotron;

Other Analyses: Capillary electrophoresis, stains and fluorimetry; tests for chirality.

Subgroup 6: Candidate Biohazard Tests – Qualifiers, Contraindications, Controls, and Characterization

Robert Hawley (Chair) and David Sourdive (Co-Chair)

Task:

Preliminary identification of measurements and analyses to test for biohazards, without regard to evidence of life or life-related molecules

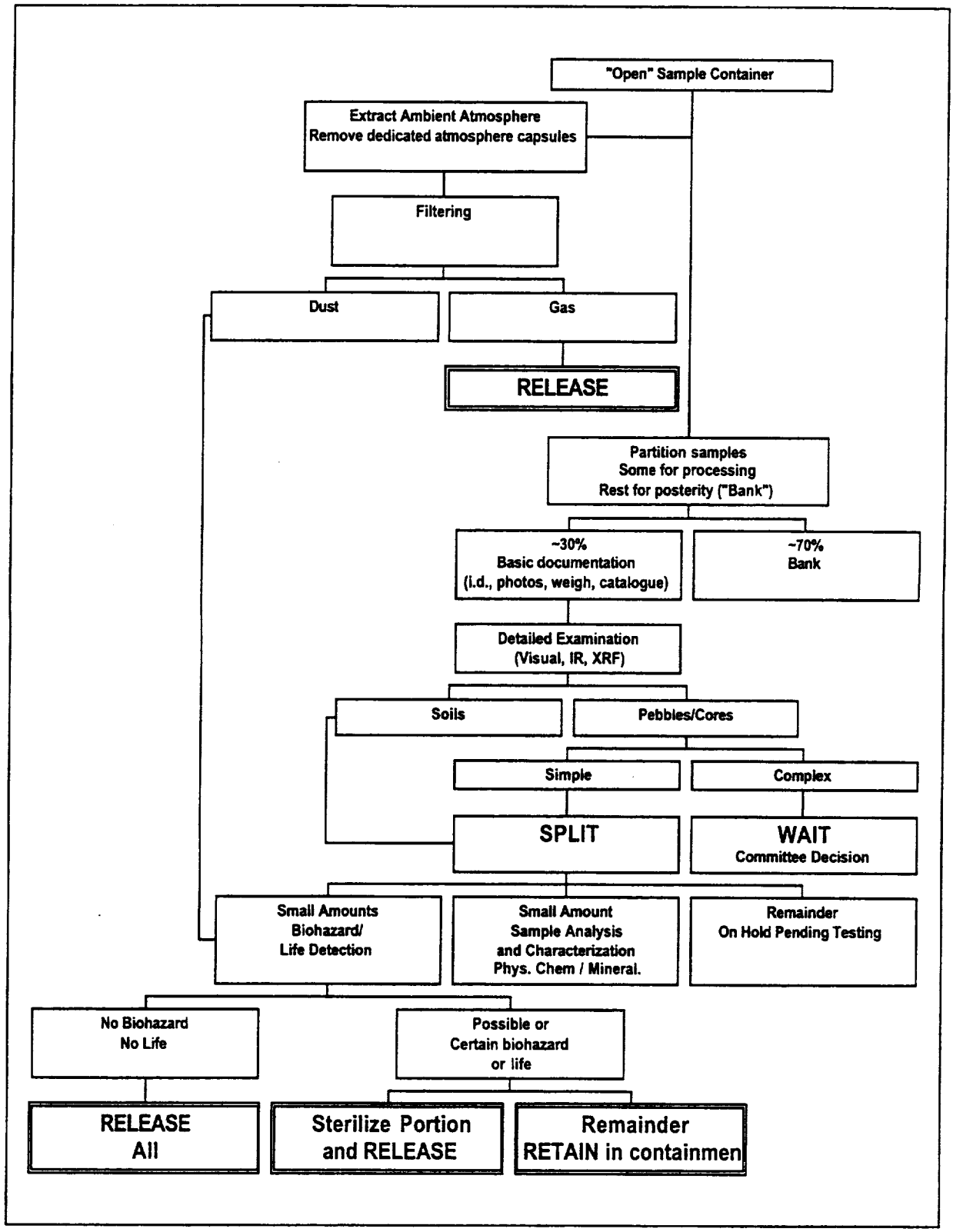
- Identify methods, test systems, and instrumentation
- Relationships of the info from complementary biohazard tests
- Anticipated problems in testing martian materials

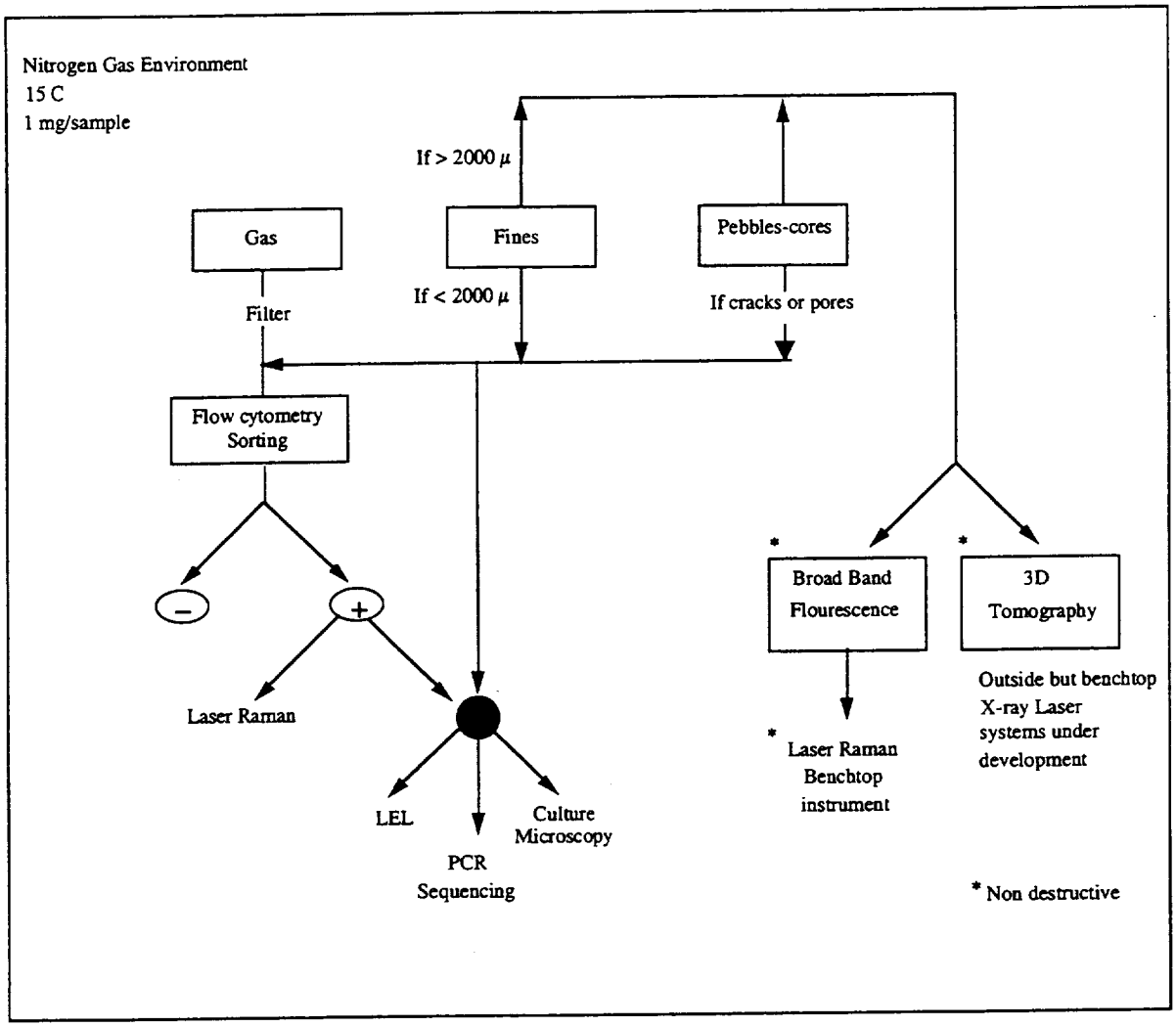
Recommendations:

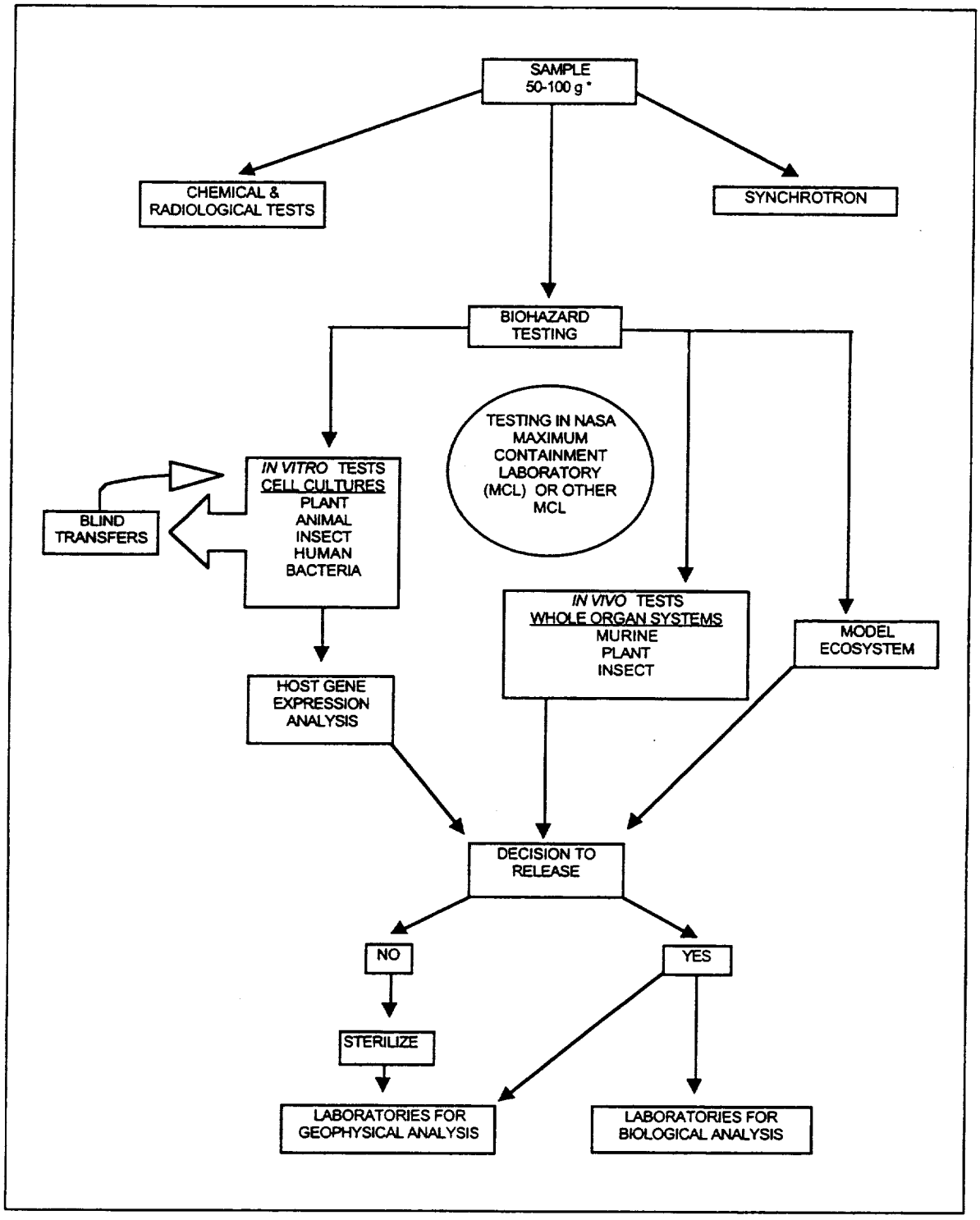
- *Preliminary testing to gather baseline information* on various sample types
  - + Descriptive and physical characteristics
  - + Comparative gas analyses
  - + X-ray imaging and 3-D image analysis for carbon analyses (syncrotron)
- *Stepwise process for biohazard analysis: in vitro and in vivo testing*
  - + *For in vitro testing*
    - – Primary and established cell lines derived from:
    - – Plants
    - – Animals
    - – Insects
    - – Humans
    - – Bacterial cell cultures
    - – Microbial community ecosystem models
  - + Focus on detecting:
    - – Replicative properties of the hazard
    - – Selected phenotypic responses
    - – Host gene expression responses
  - + *For in vivo testing: Varied model systems*
    - – Mouse (knockout mice with immune defects)
    - – Specific Pathogen Free (SPF) outbred mice
    - – Plants (Arabidopsis and others)
    - – Insect and ecosystem models (details TBD).

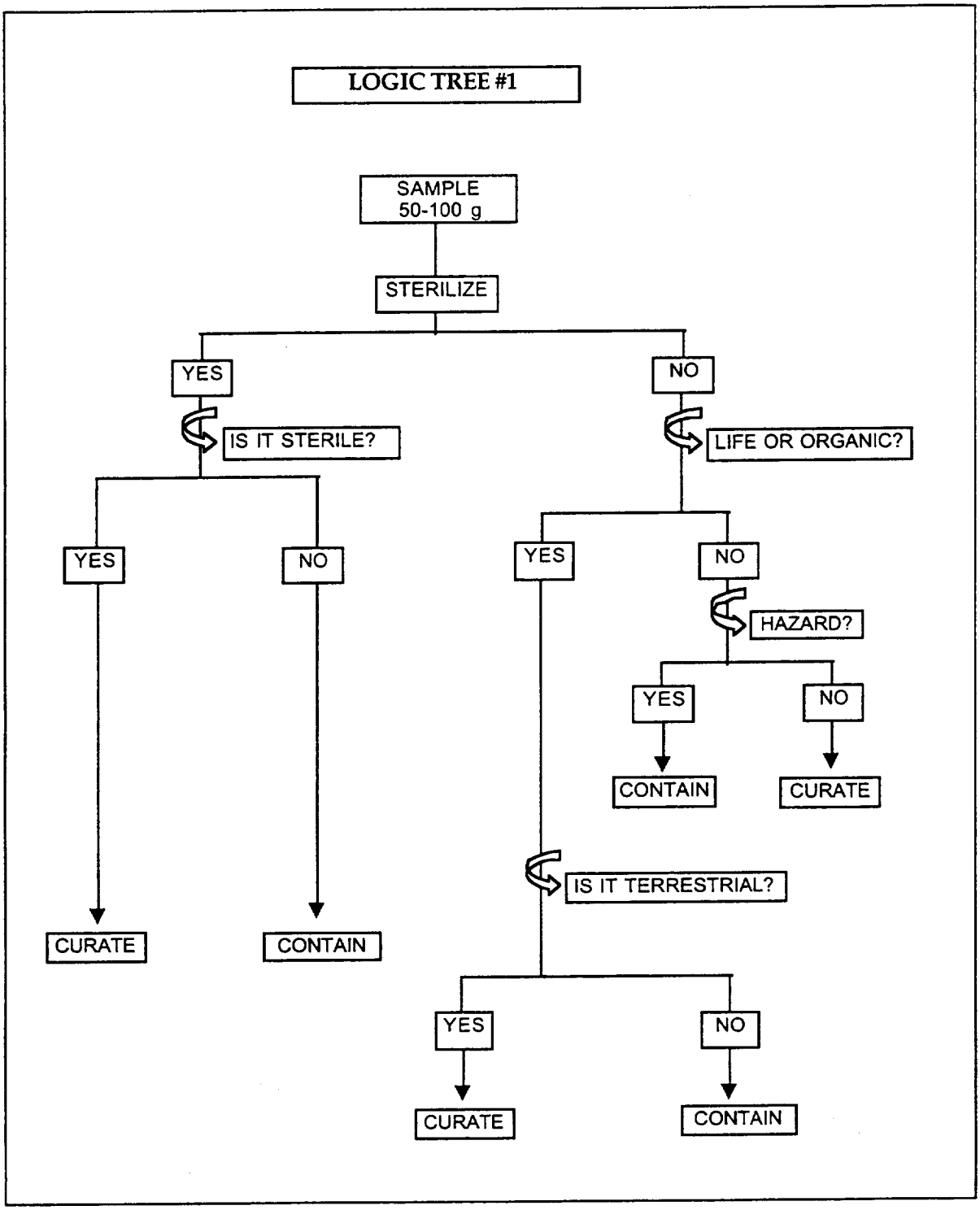
Sub-Group 6: Suggestions on Release from Containment

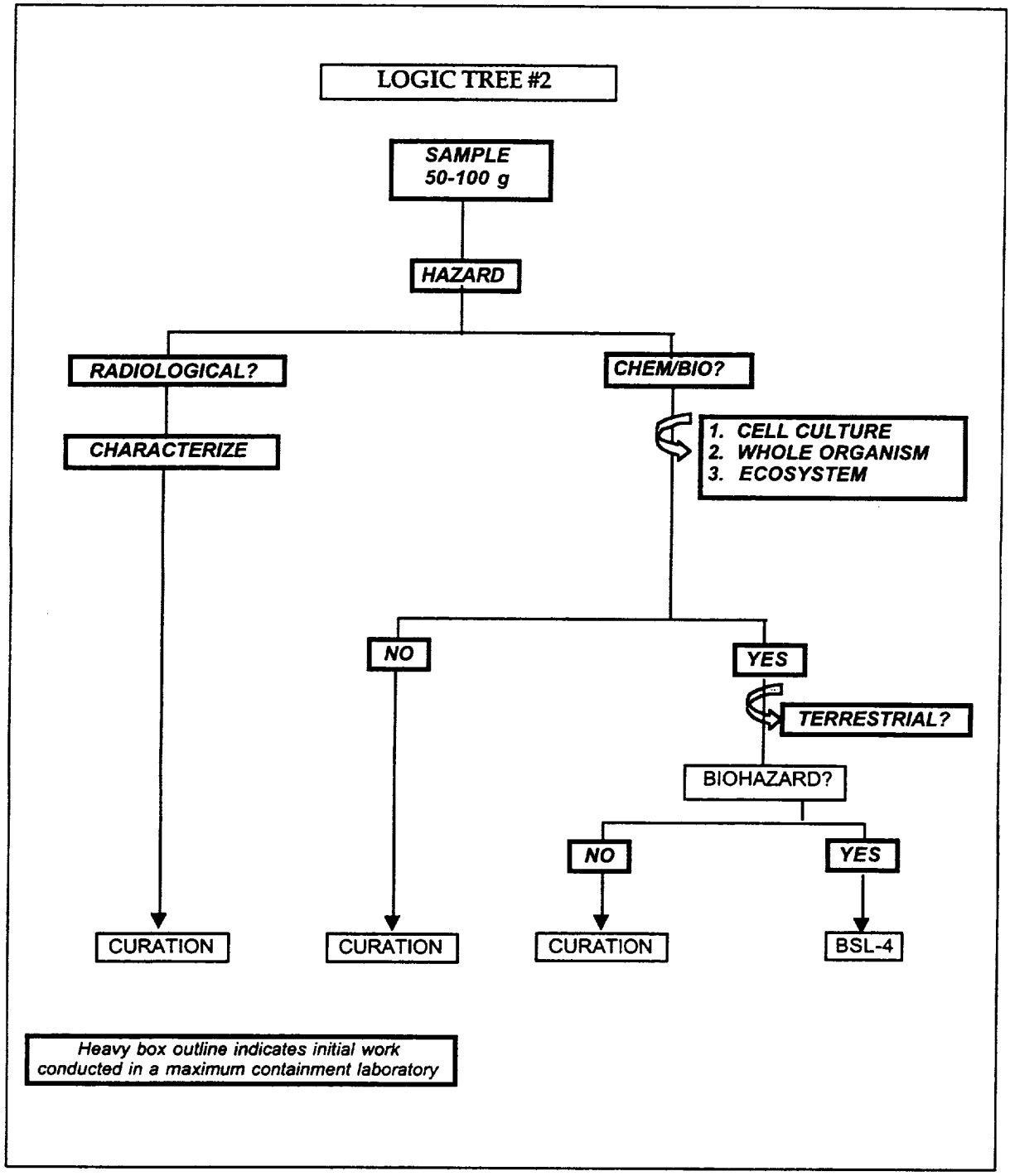
- OK to release from maximum containment if no biohazard or life form has been detected *BUT* additional experiments and life detection tests be done under BSL-3 biocontainment
- If sub-samples are released prior to completion of the protocol testing, they should be subjected to extensive gamma irradiation sterilization, with dose, time, efficacy etc. TBD
- OK to do some tests at locations other then the primary SRF (assumes maintain maximum containment & security—based on availability of adequate procedures for containing the sample, sterilizing or cleaning the outside of the sample container, and returning the sample to the containment facility).


"Open" Sample Container
Extract Ambient Atmosphere
Remove dedicated atmosphere capsules
Filtering
Dust
Gas
RELEASE
Partition samples
Some for processing
Rest for posterity ("Bank")
~30%
Basic documentation
(i.d., photos, weigh, catalogue)
~70%
Bank
Detailed Examination
(Visual, IR, XRF)
Soils
Pebbles/Cores
Simple
Complex
SPLIT
WAIT
Committee Decision
Small Amounts
Biohazard/
Life Detection
Small Amount
Sample Analysis
and Characterization
Phys. Chem / Mineral.
Remainder
On Hold Pending Testing
No Biohazard
No Life
Possible or
Certain biohazard
or life
RELEASE
All
Sterilize Portion
and RELEASE
Remainder
RETAIN in containmen


Nitrogen Gas Environment
15 C
1 mg/sample
If > 2000 μ
Gas
Fines
Pebbles-cores
Filter
If < 2000 μ
If cracks or pores
Flow cytometry
Sorting
−
+
Laser Raman
LEL
Culture
Microscopy
PCR
Sequencing
*
Broad Band
Flourescence
*
3D
Tomography
* Laser Raman
Benchtop
instrument
Outside but benchtop
X-ray Laser
systems under
development
* Non destructive


SAMPLE
50-100 g *
CHEMICAL & RADIOLOGICAL TESTS
SYNCHROTRON
BIOHAZARD TESTING
TESTING IN NASA MAXIMUM CONTAINMENT LABORATORY (MCL) OR OTHER MCL
IN VITRO TESTS
CELL CULTURES
PLANT
ANIMAL
INSECT
HUMAN
BACTERIA
BLIND TRANSFERS
IN VIVO TESTS
WHOLE ORGAN SYSTEMS
MURINE
PLANT
INSECT
MODEL ECOSYSTEM
HOST GENE EXPRESSION ANALYSIS
DECISION TO RELEASE
NO
YES
STERILIZE
LABORATORIES FOR GEOPHYSICAL ANALYSIS
LABORATORIES FOR BIOLOGICAL ANALYSIS


LOGIC TREE #1
SAMPLE 50-100 g
STERILIZE
YES
NO
IS IT STERILE?
LIFE OR ORGANIC?
YES
NO
YES
NO
HAZARD?
YES
NO
CONTAIN
CURATE
CURATE
CONTAIN
IS IT TERRESTRIAL?
YES
NO
CURATE
CONTAIN


LOGIC TREE #2
SAMPLE
50-100 g
HAZARD
RADIOLOGICAL?
CHEM/BIO?
CHARACTERIZE
1. CELL CULTURE
2. WHOLE ORGANISM
3. ECOSYSTEM
NO
YES
TERRESTRIAL?
BIOHAZARD?
NO
YES
CURATION
CURATION
CURATION
BSL-4
Heavy box outline indicates initial work conducted in a maximum containment laboratory

Mars Sample Handling Protocol Workshop #2
Bethesda, MD October 25-27, 2000

Interim Summary Report

Editors: M.S. Race, G. Kovacs, J.D. Rummel, and S. Acevedo

Objectives of the Workshop Series:

- Develop comprehensive draft protocols to assess that returned martian sample materials do not contain biological hazards
- Safeguard the purity of the samples from possible terrestrial contaminants.

Workshop #2 :

- *Used Same 12 assumptions as WS #1* (re: mission architecture, sample characteristics, containment, & SRF)
- *WS #1 findings used as basis for WS #2 discussions*
- *Organized into Sub-group tasks*

  Subgroups 1-3: *Discuss candidate tests, methods and instruments for both Biohazard and Life Detection Protocols*

  Subgroups 4-6: *Recommend specific analyses for*
  Physical & Chemical Characterization
  Molecular Biological Tests
  Organismal and Cellular Tests.

6 subgroups and Specific Tasks:

Day 1-2 (approx. 6 hours):

⇒ Life Detection (LD)
Identification and prioritization of tests and methods that could be used to detect live organisms and biomarkers in returned samples, as well as to distinguish these from terrestrial contamination and false positives.

⇒ Biohazard #1 and #2 (BH) (2 groups)
Determine if samples pose any threat to terrestrial organisms or ecosystems, whether or not samples contain life forms or non-replicative biohazards

Day 2-3 (approx. 4 hours)

⇒ Physical and Chemical Tests
Determine the physical and chemical properties of the sample that must be ascertained prior to LD or BH tests so that
a) samples can be selected for LD and BH testing and
b) LD and BH tests can be properly interpreted

⇒ Molecular Tests
Indicate and/or illustrate specific molecular tests and procedures that will be employed to accomplish the BH protocol

⇒ Organismal and Cellular Tests
Indicate and/or illustrate specific *in vivo* and *in vitro* tests and procedures that will be employed as part of the BH protocol

Life Detection Sub-group Charge:

(Norm Wainwright, Chair; Francois Raulin, Co-chair)

Identification and prioritization of tests and methods to detect live organisms and biomarkers in returned samples, as well as to distinguish these from terrestrial contamination & false positives.

- Recommended tests and methods included a combination & variety of current technologies
- Non-destructive physical methods,
- Destructive chemical and biological analyses
- Microbial culture analyses.
- Will likely be modified to include relevant future technologies.

Non-destructive methods

- **Raman, Infrared (IR), and fluorescence micro-spectroscopy**
- **Light microscopy** of fines as well as surfaces of pebbles or rock
- **Analysis of gases in the container head space**
- **Laser Desorption Mass Spectroscopy (LDMS) & Laser Raman analysis**
- **3D Tomography** applied to a totally sealed container of sample material **outside of maximum containment facilities at a synchrotron**

Destructive Chemical and Biological Analyses for LD:

- **Carbon analysis** using techniques with greatest sensitivity (progressive heating/oxidation, coupled to a Gas Chromatograph/Mass Spectrometer (GC/MS)
- **Extraction of representative samples** (initially using ultra-clean water and subsequently via organic solvent extraction)
- **Flow cytometry** to analyze single particles in the range of 2 to 100 microns
- **Culture of terrestrial microbes** using standard microbiological examination
- **Culture of martian microbes** using conditions compatible with martian life (TBD)
- **Cultures monitored and analyzed** by **simple microscopy** as well as **GC/MS** and **Liquid Chromatograph/Mass Spectrometer (LC/MS)** analyses, and **enzyme amplification methods**
- **Standard microbiological methods and selected molecular analyses** to distinguish terrestrial versus martian life
- **Enzyme amplification techniques**, including PCR, Limulus Amebocyte Lysate (LAL), adenosine triphosphate (ATP) analysis, and other methods
- **Development and use of Mars sample simulants** to test life detection methods and to train personnel.

Estimated Time and Amount for LD Tests:

- Within a 90 day period using approx. 2.5 grams of sample.

Research Advocated by LD Sub-Group on:

- **Limits of detection of all methods**
- **Validating LD methods prior to sample return**
- **Developing Mars sample simulants**
- **Refining culture conditions and analytical methods for detecting terrestrial contamination**

Biohazard Testing Sub-groups #1 & #2

(both with same sub-group charge - integrated findings)

1) Gregory Kovacs, Chair; Thierry Candresse, Co-Chair
2) David Sourdive, Chair; Margaret Race Co-Chair

Charge: How to determine if the samples pose any threat to terrestrial organisms or ecosystems, regardless of whether samples contain life forms or non-replicating biohazards.

- Built around the **use of model systems** and a decision flow chart **consistent with currently accepted biohazard testing practices.**
- **BH Testing** is considered an important **pathway toward gradual "de-containment" of the samples**
- **Working Criteria for choosing models based on probable hazard scenario** (avoid models sensitive to improbable dangers or unrealistic handling conditions). Design of protocol in consideration of relative level of harm from possible biohazards and conditions anticipated during handling/testing
- **For each model system** suggested, must **select measure of potential biohazard effect (readout) in advance**, using appropriate baseline pre-tests and negative and positive controls.
- Important to **take pre-launch data two years before sample arrival to establish both positive and negative controls** (swab samples from assembly and launch phases and test facility)
- **Present-day analysis technologies as starting point—** but recommended tests **may change prior to sample return to reflect advances in testing methodologies and practices.**

At this time, recommended BH tests include:

1. **Verification of Containment Materials Integrity**
2. **Attempt to directly culture potential microorganisms from Mars** under varying conditions
3. **Use of selected unicellular or small organisms to monitor for suitable signal readouts** (organisms and readouts: TBD)
4. Use of selected whole organisms (TBD, e.g., animals, plants, or modified organisms) **to monitor and measure selected physiologic functions, behavior, gene expression, inflammatory cascade, etc.**
5. **Use of multi-organism population tests to monitor for disruptions in complex ecosystem interactions** (Tests TBD– R&D will be required)
6. **Long term tracking of personnel, flora, and fauna in working areas** to monitor for selected physiologic parameters with 'before' and 'after' comparisons.

Estimated Amount and Time for BH Testing:
Amount in the range of **10 to 25 grams**;
Time required **~ 4 to 6 months** (most results in approx. 90 days)

Research Advocated by BH Sub-Group on:

- **Methods of generating 'control' samples**
- **Understanding how trace elements might impact analyses**
- **Study of sub-sampling procedures**
- **Development of model systems validated with terrestrial mimics of martian soil,**
- **Development of methods for sample preparation and delivery**
- **Development of phenotype databases for complex readouts in each model (e.g., global biochemical patterns, differential transcription patterns, disruptions in complex ecosystem interactions)**

BH Sub-Group also developed a detailed table for discussion at subsequent workshop.

- Rough outline of experiments (not a recommendation)
- Possible Sequence of Tests

Test Type	Procedures/Questions	Sample Usage and Timing
Verification that any potential organisms do not attack rubber, silastic, and other bio-containment materials.	Do samples affect test coupons of containment materials at various humidity levels and temperatures?	1 gram 1 - 3 months?
Input from Life Detection Protocols (discussed separately): - If life detected, this could radically change/focus the approach to biohazard testing by providing focus in terms of conditions for replication, agents that can kill the organism(s), etc. - If no life is detected, still run subsequent tests for toxicity and biohazard.	- Carbon? - C-C bonds? - Complex carbon compounds (indicative of metabolic processes)? - Skeletal remains or fossilized remnants? - Indication of live organisms (organelles, membranes, structures on microscopic evaluation)? - Life-like structures? - Living agent (replicates in environment, with coagent/host, in earth cells)? - Mutual/commensal/parasitic relationship? - Kills cells or organisms? - Kills complex multicellular organisms? - Kills everything?	TBD TBD
Multi-species infectivity, pathogenicity, toxicity testing. Look at broad host ranges (assuming that any pathogens would not be too host-specific) with well-known and standardized model systems. Use small organisms in small volumes, allowing for maximum sample conservation. Initial work all done at "BSL-4" biological containment level.	Sample preparation (rough cut): - *Crush larger clumps/rocks but do not pulverize all particulates.* - *Filter?* - *Mix into sterile water.* - *Chelate heavy metals?* - *pH buffer?* - *Use serum for some samples?* For sterilized control samples, heavily irradiate with ^{60}CO. Introduce appropriate amount (10 – 100 mg for statistical relevance) of sample to culture of unicellular organism and cell lines. Innoculate whole organisms (animals to model humans) with primary (not passaged) material. Monitor: - cell proliferation - cell morphology - deferential analyses of biochemicals - gene expression - comparative genomics (any inserted genes in host?) - reporter assays (?) - etc.	three trials + sterlized control per organism, assuming 100 mg/sample = 1.6 grams. Timing: ~ 6 months to allow for passage times.
Negative results with multiple species tests lead to downgrading to "BSL3."	In order to downgrade to "BSL-2", the following tests/criteria are proposed: > First passage from infectivity analysis (+ or –), but second and subsequent passages all negative. > DNA damage assays (mutagenesis - Ames test, strand break analysis). > Environmental damage. > Whole plant innoculations. > Diversity of growth conditions existent on Earth (extermophile, etc.) + other media. Monitor: cell viability, expression of toxic response genes.	Sample expended: ~10 – 20 grams (very rough estimate). Timing: ~6 months to allow for passage times. Note: There was good consensus on the first round (infectivity) protocol, but it was also clear that the containment level determination issues need considerably more consideration and study.
		Total = 15-25 g

Physical and Chemical Tests Sub-group

(Carl Allen, Chair; Christian Mustin, Co-chair)

Identification and prioritization of tests and methods to help select sample materials for subsequent life detection and biohazard protocols, and to facilitate their proper interpretation.

- **Endorsed for deliberations: 5-step Protocol Process and Starting Assumptions of Phys/Chem Sub-group from WS 1**
- **Specific Focus:** Determine physical and chemical properties in support of life detection and biohazard protocols. Discuss candidate tests,and identify methods and instrumentation to characterize geologic samples. Indicate amount of material and estimated time needed for specific tests.
- **Estimated Time and Amounts:**
  Time: **minutes to hours per test** (Weeks overall?)
  Amount: **30 to 100 mg per sample** for destructive analyses

Tests, Methods & Instruments selected:

1. **Initial processing** (in inert atmosphere glovebox):
   - **Sample Appearance**: Reflected light microscopy; Digital color imaging
   - **Sample Mass**: Electronic balance
   - **Major Element Composition**: Portable X-ray florescence spectrometer
   - **Sample Separation**:
     Fines – sterile spatula
     Rocks – tweezers, mechanical rock splitter

2. **Detailed Examination and Analysis**
   (performed in clean room/biosafety lab)
   - **Major/Minor/Trace Element Composition:** Inductively-coupled plasma spectrometer
   - **Mineral composition:** Petrographic microscope analysis of thin sections
   - **Inorganic/organic carbon abundances:** Acid dissolution followed by furnace pyrolysis and analysis by gas chromatograph mass spectrometer

Issues needing further consideration:

- Ensure tests conducted in a manner that introduces the least possible contamination
- Need to develop geochemical sample preparation, instruments & testing protocols that can be performed within biocontainment
- Need to address specific concerns re: impact of different sterilization methods on anticipated types of samples.
- Suggested additional analyses (not first priority)

  UV/visible fluorescence (surface organics)
  X-ray tomography (veins, vesicles, clasts)
  Energy-dispersive X-ray fluorescence (element comp.)
  Raman spectroscopy (mineralogy; organics)
  Infrared reflectance spectroscopy (minerology)
  Thermal emission spectroscopy (mineralogy)
  Mössbauer spectroscopy (iron oxide abundances)
  Environmental SEM of broken surfaces (microfossils)
  Imaging proton NMR spectroscopy (H_20)

- Additional Idea: One member advocated incubating selected samples under an artificial Mars atmosphere as an indirect indicator of active geochemistry or life.

Molecular Tests Sub-group

(Gerald Joyce, Chair; Daniel Prieur, Co-chair)

Identification of molecular biological tests and procedures for use in carrying out the biohazard assessment protocols, as well as the instruments needed to accomplish these tests.

- **Molecular tests will play two roles** in Sample Return:
  ***Primary*: Direct analysis of sample materials** to assess terrestrial contamination & assist in life detection analysis;
  Secondary: Molecular tests for analyzing biological systems that have been exposed to the returned samples.
- **Guiding Principle–** Give the sample the best opportunity to declare its biohazard potential. Special emphasis should be given to the sample handling procedures per se and whether procedures might activate or inactivate the biohazard potential of the sample.
- **Molecular biological tests to assess 3 types of deleterious effects**
  DNA damage
  Altered gene expression
  Altered levels of proteins and metabolites in response to infection or toxic exposure.
- **Premature to specify detailed molecular biological tests at this time** (Specifics dependent on changing technologies)

Use Mars simulated soils to develop an understanding of how martian samples might interfere with PCR and other proposed molecular tests.

⇒ Initially with current martian simulants

⇒ Later develop more realistic simulants with information from *in situ* chemical analyses on 2003 and 2007 Mars missions.

- **Re: Mars 2007 Mission—Suggest:**
  1) **Test robotic *in situ* sorting and concentration of soil surface materials** based on chemical analysis and microscopic visualization
  2) **Soil Drilling to assess depth of action of martian surface oxidant**
  3) **No opportunity for robotic molecular biological tests *in situ*** because of background contamination and likely false positives
- **Recommend 3 Arms to Sample Processing Protocol:**
  1) **No special processing** (extract sample with water only)
  2) **Remove materials known to interfere with PCR & other molecular tests**
  3) **Mock Procedures to mimic manipulations anticipated during scientific analyses**

Conduct appropriate **positive and negative controls.**

- Consider a **variety of pre-validated sample-processing procedures**
- **Sample processing procedures used sparingly** and only proven beneficial based on studies with control samples.

Summary: Molecular Biological Tests Sub-group

1. PCR-based assays should be used to establish background levels of biological contamination.
2. Molecular biological tests *cannot* play a major role in Mars life detection, other than to rule out terrestrial contamination.
3. Molecular biological tests will play a central role in evaluating the potential deleterious effect of Mars samples on terrestrial cells and organisms.
4. The guiding principal of biohazard analysis should be to give the sample the best opportunity to declare its biohazard potential.
5. Molecular biological tests to assess deleterious effects should focus on DNA damage, altered gene expression, and altered levels of proteins and metabolites that occur in a dose-dependent manner following exposure to the returned sample material.
6. In view of the extraordinary progress in the fields of molecular biology and genomics, it would not be prudent to specify detailed sample analysis protocols at this time.
7. Over the next five years, efforts should focus on test analyses employing martian simulants that, as a positive control, have been spiked with terrestrial DNA or other biomaterials.
8. Sample processing should be kept to the minimum necessary to obtain reliable results as determined by the analysis of positive control samples.

Organism-Based Tests Sub-group

(Jonathan Richmond, Chair; Francois Moutou, Co-Chair)

Focused on developing and prioritizing a list of specific *in vivo* and *in vitro* tests that could be used as part of whatever biohazard protocol is ultimately adopted.

Made recommendations on

- Tests, methods and instrumentation,
- Nature of the receiving facility, laboratory design,
- Specific research and development needs.

Suggested new term Planetary Protection Level (PPL)

- To categorize and describe different combinations of containment and cleanliness conditions needed within the SRF.
- Based on SRF as unique amalgam of BSL and cleanrooms
- Details TBD (see Table 1)

Equipment, Size, & Other facility related concerns:

- Need R&D to *modify equipment & procedures* not typically used in containment (Geological and Life Detection)
- *?? Size of the SRF* based on equipment requirements
- Feasibility of housing *bulkier parts of equipment outside the biobarrier ?*
- Ability to *containerize and transport materials unopened* to and from outside test equipment;
- Need to *develop robotic testing operations*
- Need to *keep equipment maximally clean and devoid of residual terrestrial organic compounds*
- *Need for multiple animal and procedure rooms* with appropriate barrier systems
- Must address *questions about when 'sterilized' materials may be removed from the SRF*
- Allowing *adequate time for commissioning* the SRF, *pre-testing* protocols, and *developing core team* competencies

Recommended Organismal/Cellular Tests:

Initial Biohazard Tests:

1) *Human cell lines and primary cell cultures*, with particular emphasis on epithelial cells
2) *Mouse cells*, with "culture-adapted" material injected into selected mice stains, and
3) *Selected microbial systems*

- *Focus on adverse effects on humans*
- *Anticipate toxic effects* on cultured cells & microorganisms *from chemical (mineral) composition of samples*
- *Appropriate controls* must be run and interpreted.

IF initial BH Tests (above) and Life Detection Tests are ALL NEGATIVE
THEN Appropriate to conduct subsequent tests under less strict containment conditions (TBD)

Tests that can be done at reduced containment include additional geophysical testing and the following recommended biohazard analyses:

1. Secondary mammalian cell culture systems.
2. Plant cell systems (Arabidopsis) and whole-plant growth experiments
3. Additional microbes and microbial systems under varying conditions
4. Horizontal and vertical transmission studies using other selected species
5. Additional experiments using a variety of techniques to test for biologically active compounds, micro-arrays (for proteins), etc.
6. Monitoring of laboratory workers throughout these studies (tests TBD), and probably for years thereafter.

Estimated Time and Amount of Material for BH Tests

Amount of Material for destructive BH tests: *TBD in consultation with biostatisticians.*
Time: Estimated *minimum of 3 months (but up to 6 months)* – assuming no positive results from life detection tests

Table 1. Anticipated Laboratory Conditions and PPL Types

PPL-type	Biocontainment	Cleanliness	'Ambient' Conditions	Used For:
PPL-α	Maximum (BSL-4)	Maximum	Mars-like (pristine)	Incoming Container and materials; some preliminary tests; sample bank / storage
PPL-β	Maximum (BSL-4)	Maximum	Earth-like	Life Detection; some Physical/Chem;TBD
PPL-γ	Maximum (BSL-4)	Moderate	Earth-like	some biohazard, some physical/chemical tests, and animal testing
BSL-3	Strict (BSL-3)	Not Clean	Earth-like	Some post-release tests TBD

Introduction to Mars Sample Handling Workshop Series: Workshop on Life Detection – Issues & Topics

John D. Rummel, NASA Headquarters

WORKSHOP ON LIFE DETECTION
ISSUES AND TOPICS

- Before martian soil and rock samples can be distributed to the research community, the returned materials will initially be quarantined and examined in a proposed BSL-4 containment facility to assure that no putative martian microorganisms or attendant potential biohazards exist.
- During the initial quarantine, state-of-the-art life detection and biohazard testing of the returned martian samples will be conducted.
- Life detection, as defined here in regard to Mars sample return missions, is the detection of living organisms and/or materials that have been derived from living organisms that may be present in the sample.

Life detection methods must be

- sensitive and comprehensive in order to preclude the untoward release of undetected putative martian biota outside the containment facility when sub-samples are distributed.
- efficient and cost-effective to maximize life detection efforts and minimize ineffective procedures that waste sample material.

In addition, applicable control methods and procedures must be developed that will distinguish unmistakable terrestrial contaminants from putative martian biota.

Geochemical analyses must be performed on sub-samples subjected to life detection assays in order to define the inherent properties of the materials returned.

Issues to be considered by workshop participants include:

- If putative martian life exists, it may somewhat resemble terrestrial life given the exchange of meteorites and potentially microbes between Earth and Mars within the inner solar system over the past 4 billion years. While divergent evolution on two worlds would likely evoke inherent differences, what fundamental, unifying properties of life as we know it should be tested, employing chemical and cellular assays that are usually used to monitor terrestrial biological activity?
- Putative martian biota may be quite different from terrestrial life, presenting morphological organization and chemical properties for which we have little or no points of reference. If so, what fundamental properties and organization (e.g., chemical changes or anomalies that suggest metabolic activity) should NASA take into account to maximize detection of putative martian life and distinguish it from false positive evidence?

Issues to be considered by workshop participants (cont.):

- Many terrestrial species survive through stages of dormancy during different life cycle stages, as well as over a range of environmental conditions. Putative martian life may likewise exhibit stages of dormancy or sporulation that could limit analytical sensitivity and overt detection. What chemical and cellular methods to detect dormant life-forms should be considered given the potential limits imposed to assay sensitivity?

Issues to be considered by workshop participants (cont.):

- We may test only a portion of the returned martian sample in the quarantine protocol, thereby preserving the remainder of the sample for subsequent research. Consequently, all life detection testing would be performed on representative sub-samples.

  (a) What geochemical and geophysical properties of the entire returned sample must be taken into account to select representative sub-samples? What are the final criteria for sub-sample selection and preparation?

  (b) Are there other procedures and methods that have potential applications to Mars samples and at the same time preserve the properties of soil and rocks for future planetary geology research? Should NASA invest in research into the potential applications of these emerging procedures?

Issues to be considered by workshop participants (cont.):

- In light of anticipated difficulties in the detection of putative martian life (e.g., potentially dormant forms or putative microbes existing as a low biomass in the sample), how many representative sub-samples should be tested in the quarantine protocol? What is the acceptable margin of error that will assure NASA has not missed detecting putative martian life?
- At prior quarantine protocol workshops, several participants with expertise in terrestrial microbial life detection have emphasized a high priority requirement for sensitive chemical analyses if NASA is ever to detect a low biomass or dormant putative martian biota. In addition to fundamental elemental chemical analysis of representative sub-samples, what chemical methods should receive a high priority? What methods and procedures should be considered to reduce the margin of error? What type and number of controls will be needed to definitively distinguish potential terrestrial contamination?

Issues to be considered by workshop participants (cont.):

- If putative martian microbes exhibit recognizable morphological characteristics, what cell biology methods (e.g., electron microscopy, flow cytometry. etc.) provide the highest-ranking priorities for life detection, even though putative martian biota may exist in a low biomass or in dormant forms? What methods and procedures should be considered to reduce the margin of error? What controls will be used to definitively distinguish potential terrestrial contamination?
- And for final discussion: If life is detected in the sample (other than confirmed terrestrial contamination), what are the next steps?

Workshop #3 Process - Organization

John D. Rummel, NASA Headquarters

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 3

SUB-GROUP CHARTERS & MEMBERS

3 Sub-Groups on Day 1

Sub-Group 1A:

What fundamental, unifying properties of life as we know them may be applied to life detection on martian soil and rock sub-samples, employing the utility of chemical and cellular assays usually exploited to detect or monitor terrestrial biological activity?

Kenneth Nealson (Chairperson)
David J.D. Sourdive (Co-Chairperson)
Gregory T.A. Kovacs
David A. Relman
Mitchell L. Sogin
Andrew Steele
Michel Viso
Norman Wainwright
Mohan Wali

Sub-Group 1B:

If putative martian biota are quite different from terrestrial life, what fundamental morphological organization and chemical properties should be taken into account to maximize future life detection efforts? Are there emerging methods and procedures (e.g., X-ray microscopy) that should be considered?

John Baross (Chairperson)
Jacques Grange (Co-Chairperson)
Jeffrey L. Bada
J. Gregory Ferry
Marilyn Fogel
Joseph B. Lambert
Christian Mustin
Arthur B. Pardee

Sub-Group 1C:

What geochemical and geophysical properties of the entire returned sample must be taken into account to select representative sub-samples? What are the final criteria for representative sub-sample selection and preparation? Approximately how many representative sub-samples may be tested?

David Blake (Chairperson)
Jean-Pierre Bibring (Co-Chairperson)
Carl Allen
David Beaty
Geoffrey Briggs
David Lindstrom
John J. Nicholaides III
Michael J. Singer
Alan H. Treiman

MARS SAMPLE HANDLING PROTOCOL WORKSHOP 3

SUB-GROUP CHARTERS & MEMBERS

2 Sub-Groups on Day 2

Sub-Group 2A:

What are the ranking priorities for sensitive chemical methods to enable detection of low biomass or dormant putative martian biota? What applications of these particular methods render their applicability and reduce the margin of error? What type of controls will be necessary to definitively distinguish potential putative extraterrestrial life from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

Jeffrey L. Bada (Chairperson)
Christian Mustin (Co-Chairperson)
Carl Allen
John Baross
David Beaty
Jean-Pierre Bibring
Geoffrey Briggs
Jacques Grange
Joseph B. Lambert
J. Gregory Ferry
Marilyn Fogel
John J. Nicholaides III
Arthur B. Pardee
Mitchell L. Sogin

Sub-Group 2B:

What are the ranking priorities for sensitive cell biology methods that will enable detection of low biomass or dormant putative martian biota? What methods should be considered to reduce the margin of error? What controls are warranted to be able to definitively distinguish putative martian life and its morphology from terrestrial contamination? What equipment will be necessary? Indicate the estimated amount of sample that will be required. How much time will be needed to conduct each particular test? Indicate whether testing can be done inside or outside the proposed BSL-4 containment facility.

Norman Wainwright (Chairperson)
Michel Viso (Co-Chairperson)
David Blake
Gregory T.A. Kovacs
David Lindstrom
Kenneth Nealson
David A. Relman
Michael J. Singer
David J.D. Sourdive
Andrew Steele
Alan H. Treiman
Mohan Wali

APPENDIX E: REFERENCES

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APPENDIX F:
GLOSSARY OF TERMS AND ACRONYMS

ALH	Alan Hills (Antarctica)
ATP	Adenosine Triphosphate
BSL	Biosafety Level
CAPTEM	Curation and Analysis Planning Team for Extraterrestrial Materials
CDC	Center for Disease Control (U.S.)
CNES	Centre National d'Etudes Spatiale (French)
CNRS	Centre National de la Recherche Scientifique (French)
COMPLEX	Committee on Planetary and Lunar Exploration
DNA	Deoxyribonucleic Acid
Eh	Oxidation Potential
EPA	Environmental Protection Agency (U.S.)
ESI	Electrospray Ionization
GC/MS	Gas Chromatograph/Mass Spectrometer
HEPA	High Efficiency Particulate Air (filter)
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IR	Infrared
LAL	Limulus Amebocyte Lysate
LC/MS	Liquid Chromatograph/Mass Spectrometer
LD/MS	Laser Desorption Mass Spectroscopy Materials
LIFARS	Laboratory Instrument for Analysis of Returned Samples
MALDI	Matrix Assisted Laser Desorption/Ionization (Mass Spec)
MS	Mass Spectroscopy
MSHARP	Mars Sample Handling and Requirements Panel (U.S.)
MSHP	Mars Sample Handling Protocol
NAS	National Academy of Science (U.S.)
NASA	National Aeronautics and Space Administration (U.S.)
NASA-CP	NASA Conference Proceedings
NIH	National Institutes of Health (U.S.)
NMR	Nuclear Magnetic Resonance
NRC	National Research Council (U.S.)
OC	Organic Carbon
PCR	Polymerase Chain Reaction
pH	Measure of hydrogen ion concentration (acidity)
PHB	Polyhydroxybutyric Acid
PP	Planetary Protection
RNA	Ribonucleic Acid
'riffle splitter'	A mechanical separation device used for geological samples
SEM	Scanning Electron Microscopy
SPF	Specific Pathogen Free
SRC	Sample Return Canister
SRF	Sample Receiving Facility
SSB	Space Studies Board

TBC	To Be Confirmed
TBD	To Be Determined
TEM	Transmission Electron Microscopy
USDA	U.S. Dept of Agriculture
UV	Ultraviolet
WHO	World Health Organization
'witness plates'	Controls for forward contamination; used to monitor for bioload on spacecraft.
XRD	X-ray Diffraction
XRF	X-ray Fluorescence (Spectrometer)

REPORT DOCUMENTION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2001	Conference Publication

4. TITLE AND SUBTITLE

Mars Sample Handling Protocol Workshop Series: Workshop 3

5. FUNDING NUMBERS

896-50-02-01

6. AUTHOR(S)

Rummel, John D., Margaret S. Race, Kenneth H. Nealson, Sara E. Acevedo, Editors

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Ames Research Center
Moffett Field, CA 94035-1000

8. PERFORMING ORGANIZATION REPORT NUMBER

A-00V0051

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

National Aeronautics and Space Administration
Washington, DC 20546-0001

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

NASA/CP—2001-211388

11. SUPPLEMENTARY NOTES

Point of Contact: Sara Acevedo, Ames Research Center, MS 245-1, Moffett Field, CA 94035-1000
(650) 604-4223

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified-Unlimited
Subject Category 88
Availability: NASA CASI (301) 621-0390

Distribution: Standard

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 words)***

This report provides a record of the proceedings and recommendations of Workshop 3 of the Series, which was held in San Diego, California, March 19-21, 2001. Materials such as the Workshop agenda and participant lists as well as complete citations of all references and a glossary of terms and acronyms appear in the Appendices. Workshop 3 builds on the deliberations and findings of the earlier workshops in the Series, which have been reported separately.

During Workshop 3, five individual sub-groups were formed to discuss the following topics:

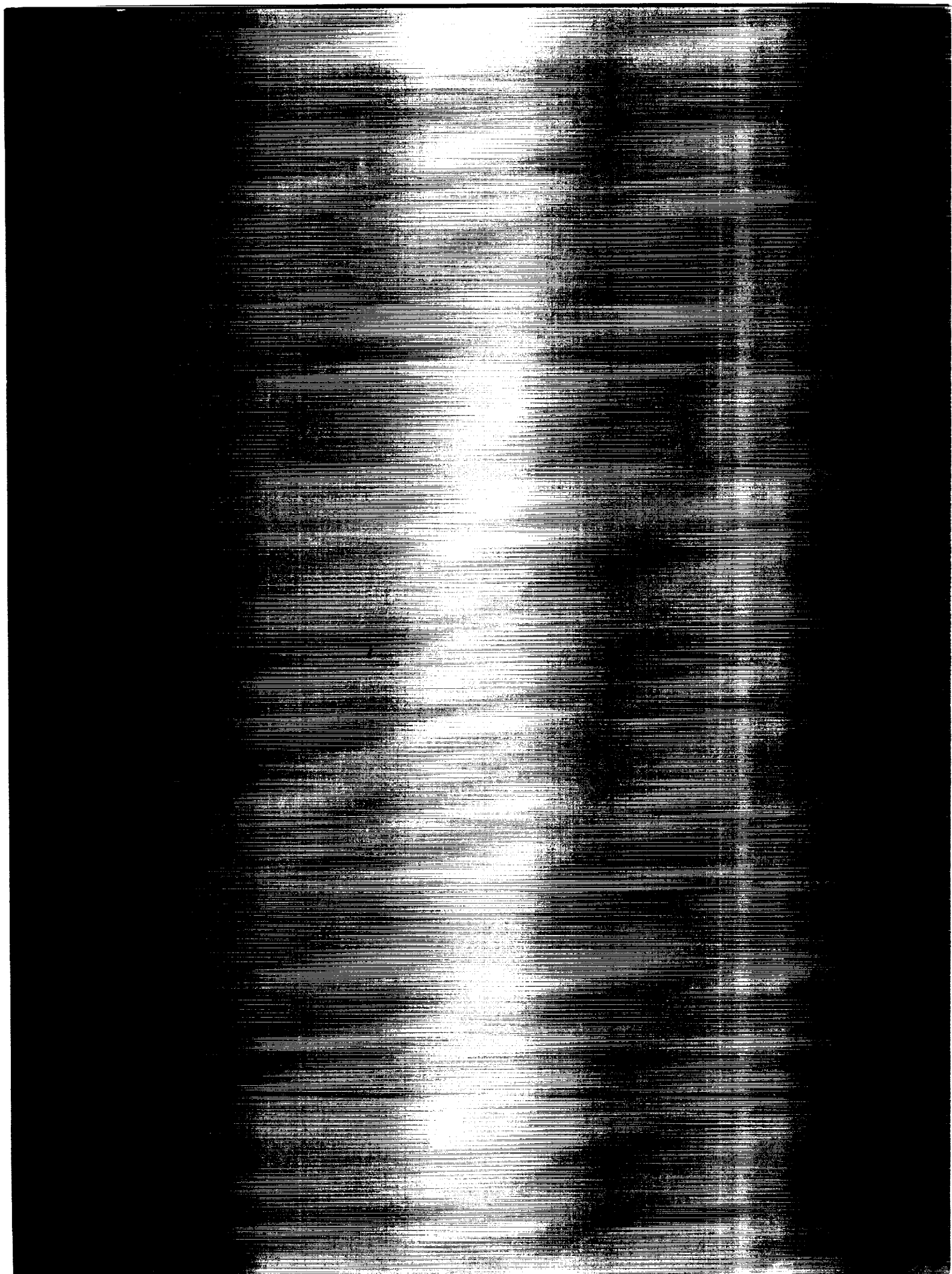
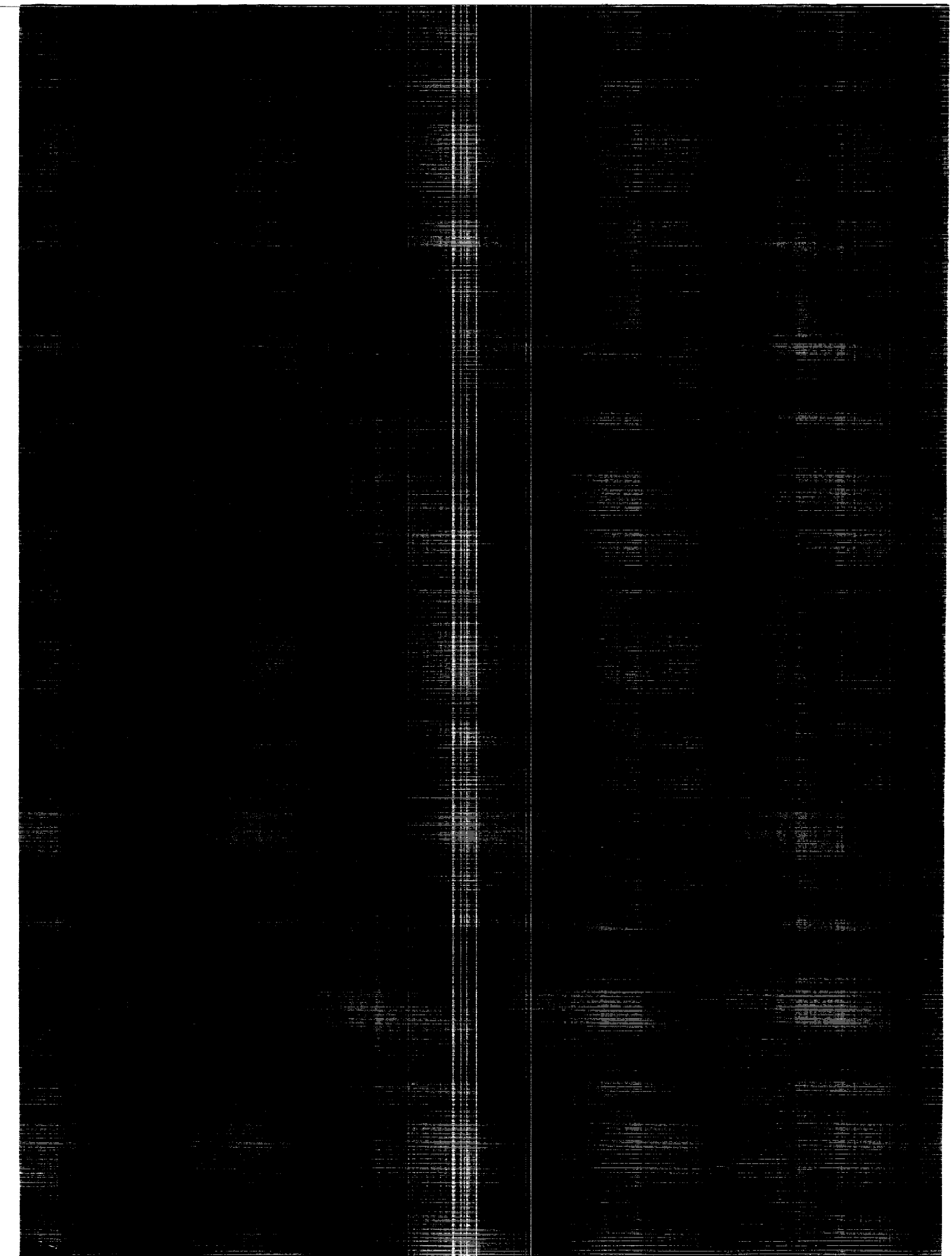
- Unifying Properties of Life
- Morphological organization and chemical properties
- Geochemical and geophysical properties
- Chemical Methods
- Cell Biology Methods

14. SUBJECT TERMS

Mars Sample Return, Planetary Protection, Sample Handling Protocol

15. NUMBER OF PAGES

134

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified