METHODS OF RESEARCH ON INFECTIOUS DISEASE IN SPACEFLIGHT

A PRELIMINARY STUDY OF APPLICATION OF BIOSATELLITE TECHNIQUES TO STUDIES OF INFECTIOUS DISEASE PROCESSES

CONTRACT NASW-2073

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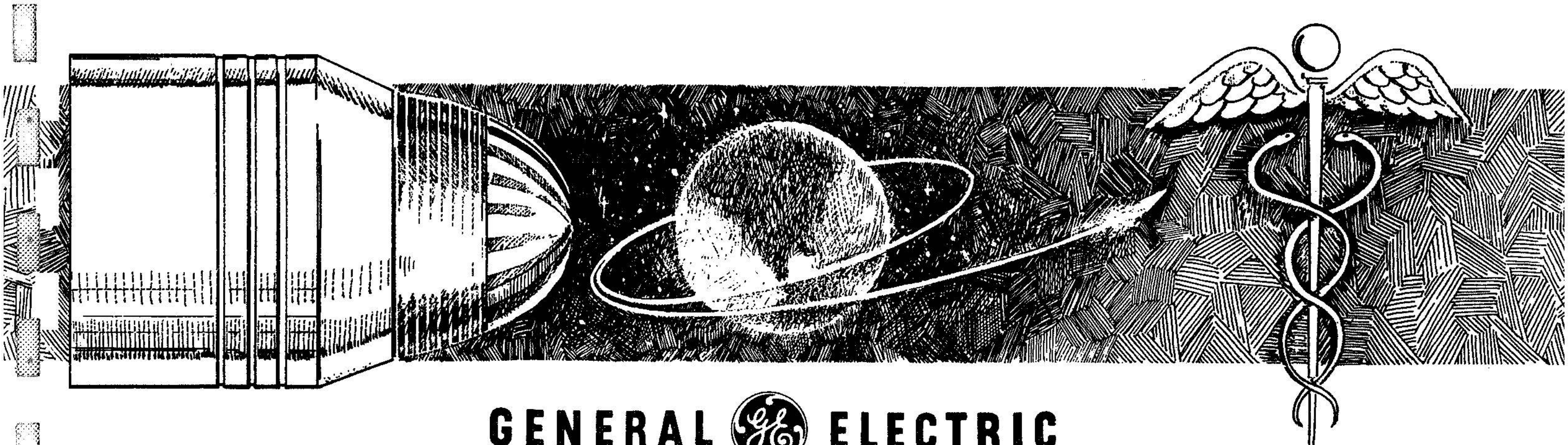

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I. INTRODUCTION AND SUMMARY

A preliminary study has been performed, under the auspices of the Office of Space Science and Applications of NASA, of the report entitled "Infectious Disease in Manned Spaceflight: Probabilities and Countermeasures", prepared by the Panel on Microbiological Problems of Manned Space Flight, John Spizizen, Chairman, Space Science Board, National Academy of Sciences - National Research Council, 1970. This study builds on the experience obtained on the Biosatellite program to place bounds on the feasibility of the proposed space experimentation.

The purpose of this study was to develop a preliminary definition of the types of research programs needed to implement the Space Science Board's recommendations with emphasis on a preliminary characterization of the programs as suited to analysis, conventional ground-based research, space simulation studies, and manned or unmanned spaceflight research programs. Research objectives were assigned to one or more of these categories on the basis of technical feasibility at the least projected cost. In keeping with the concerns raised by the Space Science Board, particular care was exercised in assigning microbiological or immunological experiments to manned spacecraft to preclude significant chance of hazard to the crew.

The Board was quite explicit in its cautions concerning the resistance of the crew to infection, even by agents not normally considered pathogenic. In view of the system complexity inherent in confining airborne pathogens within animal quarters, and the potential for undetected accidental release of the organisms into the spacecraft atmosphere and life support system, pathogenic microorganisms and large populations of viable non-pathogens should be employed in space research only on unmanned spacecraft until such time as any changes in crew resistance are firmly established. Consequently, space experiments employing pathogens have been assigned to unmanned spacecraft in this study.

Many of the research objectives proposed by the Space Science Board can be accomplished in ground-based laboratories or by analysis at relatively low cost. Studies of the response of local and specific immune defenses of the host to prolonged spaceflight environments generally require the involvement of man in actual spaceflight due mainly to the complexity of measurements and observations involved. However, complete studies of phagocytic action and of specific immune response require use of virulent microorganisms; therefore a portion of this study has been assigned to unmanned systems. Epidemiological studies also require virulent organisms and consequently they too require unmanned systems.

The experimental animals employed in those studies to be performed on manned spacecraft may be quite varied as the result of the types of measurements to be made, but those experiments assigned to unmanned missions would probably require only two species, the mouse for epidemiological and latent or slow virus experiments, and a somewhat larger animal, such as chickens or guinea pigs for studies of immune responses. Consequently, two basic types of experiment hardware could be employed for multiple use for the unmanned programs, both compatible with Biosatellite-type spacecraft.

It must be emphasized that we have no spaceflight experience with the small animals used for this type of study, and we can anticipate a significant learning process before we understand how to maintain them in suitable condition for studies of infectious disease processes. It appears that flight programs will be required in which their physiological response to long term space flight is examined before we look for more subtle changes associated with resistance to infection.

Although the magnitude of the experimental programs is not yet established with any degree of accuracy, the scope of the Space Science Board recommendations is so broad as to make the need for an assessment of program priorities obvious, both from the standpoint of need by manned programs and by virtue of their value to medical practice. Correspondingly, a detailed analysis of the experiment requirements is required to establish realistic costs for the various recommended programs.

II. ANALYSIS OF IMPLEMENTATION METHODS

In evaluating the means by which the recommendations of the Space Science Board may be implemented, the following ground rules were applied.

1) The least costly means of satisfying the scientific needs expressed in the recommendations was selected according to the following scale from least to most costly: Analysis, conventional ground-based laboratory investigation, ground-based flight simulation in a closed environment, space flight (manned or unmanned).

2) The potentially greater flexibility and capability of manned spaceflight experimentation over unmanned spacecraft biological experiments mandates the assignment of biological experiments to manned systems except where the experimental requirements are incompatible with the spacecraft or its mission from the standpoints of hazard both to the experiment validity, and to the manned spacecraft or its crew.

3) Viable pathogens, virulent or attenuated, are not acceptable agents for use in studies on manned spacecraft because of the potential hazard to the crew which could affect their capacity to perform in critical situations.

4) Experimental animals should be employed for studies of specific immune responses using vaccines prior to similar experimentation with man.

5) Large populations of viable microorganisms are to be avoided on manned spacecraft because of the potential disruption to the normal flora of the astronauts if they were accidently released in the spacecraft environment.

Table 1 summarizes the means selected to implement the Space Science Board recommendations. The individual recommendations for research are discussed on the following pages, numbered in the same manner as in the Space Science Board report.

TABLE 1. SUMMARY OF METHODS FOR IMPLEMENTING SPACE SCIENCE BOARD RECOMMENDATIONS FOR RESEARCH ON INFECTIOUS DISEASE IN SPACE FLIGHT

RECOMMENDATION	ANALYSIS	CONVENTIONAL LABORATORY	SPACE-SIMULATION CHAMBER	MANNED SPACECRAFT	UNMANNED SPACECRAFT
3a. Viability and behavior of Infectious Aerosols*					
• Aerosol stability	X				
• Aerosol deposition		X			
• Aerosol generation from surfaces		X			
• Penetration of aerosols into lungs.	X				
3b. Longevity of Microbial Aerosols in Weightlessness					
◦ Viability		X**			
• Virulence		X**			
3c. Epidemiological Studies			X		X
3d. Response to Vaccine					
◦ Inactivated			X	X	
◦ Live			X		X
3e. Effect of Prolonged Spaceflight on Local Host Defenses					
• Phagocytic Clearance from Lungs				X	X
• All Others				X	
3f. Effect of Prolonged Spaceflight on Specific Immune Mechanisms					
◦ Processing antigens by macrophages				X	X
• All Others				X	
3g. Repopulation of GI flora		X			
3h. Latent and slow virus infection in spaceflight					X

* Viability considered in (3b.)   ** If proved feasible - see text.

TABLE 1. (CONTINUED)

RECOMMENDATION	ANALYSIS	CONVENTIONAL LABORATORY	SPACE-SIMULATION CHAMBER	MANNED SPACECRAFT	UNMANNED SPACECRAFT
3i. Host Factors Affecting Latent Infection		X			
3j. Changes in Microbial Flora					
◦ Characterization			X	X	X
◦ Selection of Virulent Agents					X
3k. Effects of Isolation on Susceptibility to Infection			X		

3a. Determination of the viability and behavior of representative infectious aerosols in atmospheres of altered gas content and pressure. These studies should include not only the atmospheres planned for spaceflight, but also further digressions from normal in order to detect effects that are potentially more significant and that would not be apparent in less extreme conditions.

3b. Determination of the longevity of microorganisms in various sizes of aerosolized particles under weightless conditions in various atmospheres. Initial studies can utilize simulated zero-gravity chambers, e.g., a revolving torus; definitive experiments should be performed in spaceflight under actual weightlessness.

These two recommendations may be considered as one in that the same experimental studies can attack both questions effectively, and in fact, information from the implementation of one would be directly applicable to the other. A program devoted to fulfilling the recommendations would logically address physical behavior of the aerosol on one hand, and viability and virulence on the other.

1) <u>Physical behavior of the aerosol</u>

a) <u>Behavior of particles in a gaseous suspending medium</u>

The behavior of viable bacterial aerosols in quiescent and flowing gas has been studied experimentally and the data have been shown to agree quite well with theory for particles from one to 30 μ in diameter, both for turbulent and for laminar flow cases. It appears that the influence of gravity on the physical stability of the aerosol is readily describable analytically and should not of itself require space flight experimentation.[1]

b) <u>Deposition of particles on surfaces</u>

The nature of the forces which cause microorganisms to be attracted to and adhere to surfaces has not been described satisfactorily.

b) Continued

The magnitude of the forces varies depending on the magnitude and sign of electrical charges on the surfaces relative to those carried by microorganisms. Consequently, under certain circumstances, gravity may play a major role in deposition of particles from an aerosol, while in other cases only a minor role.

Experimental programs such as that described by Enlow[2] are being directed toward identifying and quantifying the parameters which govern aerosol depositions. However, the magnitude and distribution of electrical charge on a spacecraft, particularly one in deep space, is not well defined. Consequently, space flight experimentation may be required to finally assess the interaction of the physical forces involved. For the most part, the response of the microbial aerosol to the physical factors affecting deposition can be elucidated by ground-based experimentation.

c) <u>Aerosol generation from surfaces</u>

The factors affecting adhesion of bacteria to surfaces can be addressed in the ground-based laboratory because the adhesive forces involved are generally far in excess of gravitational forces so that gravity becomes of negligible influence (Enlow[2]).

d) <u>Penetration of aerosols into lungs</u>

As described above, the theoretical description of the transport of aerosol particles in a gaseous stream is on firm ground. Consequently, analytical definition of the influence of gravity

d) Continued

on deposition of aerosol particles in the lungs appears tractable. Limited experimentation in space flight should verify the analysis and could be performed as a part of an epidemiological study with experimental animals.

2) <u>Aerosol viability, longevity and virulence</u>

Short-term evaluations of the viability or virulence of aerosolized particles and the death rate of microorganisms contained therein may be performed in earth-based laboratories. The duration of such experiments are limited by the size of the aerosol particle and hence its settling rate, and the size of the experimental chamber. One can expect to maintain the aerosol from a few minutes to several days depending on the above-mentioned conditions.

Long-term evaluations of viability and virulence depend on removing gravitational influences. Because an airborne particle tends to move somewhat independently of its container, gravitational settling cannot be prevented by devices such as revolving containers. By opposing settling with a constant flow of air or other gas, one might maintain an aerosol for prolonged periods. This possibility should be investigated thoroughly before embarking on a space flight program to obtain the physical stability required to evaluate viability.

3c. Performance of epidemiological experiments, in simulated and actual flight, on the transmission of respiratory agents in relation to (i) number of persons or animals in the space chamber, (ii) their state of susceptibility or resistance, (iii) degree of confinement, i.e., space per unit animal or man, (iv) number of microorganisms released into the environment by the infected persons, (v) type of aerosol produced, and (vi) enhancing effect of sneezing, coughing, talking.

Most of the above listed parameters can be studied in ground-based laboratories and simulated spacecraft, providing the effect of gravity, if any, on the dissemination and stability of aerosols can be accounted for either analytically or experimentally. The influence of population density, degree of confinement, number of microorganisms released into the environment by the infected persons, the type of aerosol produced, and the enhancing effect of sneezing, coughing and talking can all be established in properly-designed ground-based experiments with fair assurance of translation of the results to the space flight environment, providing a base-line response for one or more condition in the operating spacecraft is established.

The major parameter to be studied in actual space flight is a possible unique change in the state of susceptibility of man or animal as a result of physiological changes occurring in response to weightlessness or changes in biological rhythms. A flight program to study these changes involves as a minimum: (1) challenging experimental subjects with infectious agents under a limited number of conditions and investigating their susceptibility to infection as a function of space flight duration, (2) evaluating subsequent transmission of the disease to other experimental subjects, (3) examination of the experimental subjects to verify involvement of the infectious agent under study, and (4) suitable ground-based controls.

Epidemiological studies of this type generally employ large animal populations and require careful statistical design. To minimize the number of experimental animals used in flight tests, an abnormally large ground-based population must be studied to establish the norm as firmly as practically possible, and rigorous statistical design must be employed. To maximize the data return per spacecraft, the mouse appears to be the logical candidate, partly because of its small size and partly because of the extensive backlog of data on transmission of airborne infectious agents using this animal as a model.

The need to use virulent infectious agents for the flight experiments tends to mandate against use of manned systems and toward an unmanned space laboratory such as Biosatellite or a recoverable Biological Explorer. By appropriate formulation of the experimental protocol, the experiment can also address the question of penetration into the lungs by aerosols discussed above.

Translation of the data obtained with the mouse model to man in similar circumstances probably requires additional ground-based studies using man as the experimental subject. Insofar as possible, sufficient microbiological characterization of manned spacecraft during missions should be performed to enhance the recovery of data related to actual transmission of infection between crew members which may occur unintentionally.

3d. Determination of the course of response in chamber tests or in flight, to a live or inactivated vaccine with which the recipient has had no previous experience, e.g., Brucella, tularemia or cholera vaccines, or live adenovirus type 4 oral vaccines.

Ground-based chamber tests with live oral vaccines may be useful to evaluate the effects of changes in microbial flora on the immune response of the host, but the effect of long-term weightlessness and changes in periodicity on the hosts capability to respond normally to antigenic stimulus requires space flight experimentation.

Studies of the immunological response of man to vaccines as described above should not be performed in prolonged space flight until after animal experimentation has been performed to preclude significant possibility of severe abnormal response to vaccination.

The experimental animal chosen for these studies should be large enough to permit repeated sampling of blood, but small enough to permit reasonably large numbers of specimens to be included in a space flight experiment. Of those animals that have been proposed for space flight experiment, the chicken (U. of Cal., Davis) may be most suited because of extensive data available on the immune response of that animal, for example to Newcastle's Disease Virus, and because of an apparently viable long-term canulation technique which can be used for remote blood sampling.

Many of the studies can be performed using manned spacecraft provided that the antigens employed are inactivated. Because the final measure of the immune response is the degree to which it protects against development of clinical disease symptoms, it would seem to be necessary to perform such studies with virulent pathogens. Consequently, the most effective research program in this area would involve experimental investigations on both manned and unmanned spacecraft. In the case of unmanned spacecraft, specimen recovery would be required.

3e. Determination of the long-term effects of spacecraft environmental factors (particularly weightlessness, altered atmospheres, stress, and noxious substances) on local host defense mechanisms. Particular attention should be given to mucociliary function, phagocytic clearance of organisms from the lungs, lysozyme content of secretions and blood, drainage of nasal sinuses, secretion of immune globulin A (IGA) into respiratory and gastrointestinal tracts, and peristalsis.

Because of the complexity of sampling and measurement of most of the local host defense mechanisms, it appears that these studies can best be performed on manned spacecraft using non-viable antigenic material and foreign particulate matter to test the response of these mechanisms. The participation of man in the experiments appears to be vital to their feasibility.

The measurement of lysozyme secretions could be performed conveniently using man as the experimental subject, but space experiments on most of the other mechanisms would require experimental animals. Each of the local immune mechanisms represents a field of specialty in itself, involving many potential experimenters employing a variety of experimental animals. Experimental programs must be devised which permit study of these mechanisms in as few mammalian species as possible, thereby allowing use of one set of experimental animals for study of several of the mechanisms on the same flight mission.

As the combined effect of all of the host's defense mechanisms will be evaluated as a part of epidemiological studies, a decision to follow the above experimental programs with a study of the response of the local mechanisms to viable infectious agents can be deferred until it is determined if the epidemiological results can be correlated with experimental results from the detailed studies of host defenses using non-viable antigenic and particulate materials. One possible exception to this position requiring further evaluation is the study of phagocytosis where the virulence of the invading organism plays a strong role in determining the effectiveness of the phagocytic action. In this case, where a delicate balance exists between the capabilities of the phagocyte and the invading pathogen, minor impairment of the host's defenses by physiological derangement may have marked consequences on the progress of infection. Studies to evaluate this possibility would require unmanned systems due to the need for employing virulent agents. Studies of phagocytic clearance of microorganisms from the lungs might best be accomplished in the same or similar spacecraft configuration used for epidemiological studies, requiring in-flight exposure to the aerosolized infectious agent and recovery of preserved specimens.

3f. Investigation of the effects of prolonged spaceflight on specific immune mechanisms. Studies should emphasize processing of antigens by macrophages, response of lymphocytes to invading pathogens, function of the thymus, transformation of lymphocytes to antibody-producing cells, and synthesis of immune globulin.

As in the case of local defense mechanisms, most of the specific immune mechanisms are subject to study using non-viable antigens, and therefore are suited to manned spaceflight experimentation for which the collection, preservation and analysis of blood specimens is simpler and more reliable than on unmanned systems. Again the exception lies in the study of phagocytosis which requires viable pathogens for reliable characterization of the capabilities of that mechanism as a function of flight conditions and duration.

The particular requirements of these studies include means for inoculating the experimental animals and for repeatedly withdrawing blood samples for analysis or preservation. Much of this burden can be borne by the crew in manned systems employing techniques common to the ground-based laboratory. Unmanned systems will require cannulation of experimental animals or periodic sacrificing and preservation of groups of small animals (such as mice). In either case, recovery of specimens will be required for post-flight analysis.

3g. Development of means to repopulate the gastrointestinal flora in the event that broad-spectrum chemotherapy becomes necessary on long-duration spaceflights.

Upon first examination, it would appear that this task could be performed entirely in ground-based laboratories and simulated space environments. Certainly the development of the inoculum and methods of application can be performed in that manner. Our inability to answer the question posed by the Committee on p. 45 "Can the space environment so effect host immune mechanism as to permit microbial agents considered as nonpathogenic, or normal flora, to produce illness?" will force an evaluation of the need to test repopulation techniques on experimental animals in long-term space flight. The selection of manned or unmanned spacecraft will depend on our understanding of the nature and behavior of the microbial flora of closed systems.

3h. Examination of the effects of the spaceflight environment on latent and slow virus infections experimentally induced in animals.

In keeping with the need to avoid potential hazards to the spacecraft and crew, the examination of latent and slow virus infections should be performed on unmanned spacecraft. In establishing rates of infection or lethality as a function of dose, large numbers of experimental animals are required. This factor, together with the many permutations and combinations of environments, mandate an extensive ground-based control program and small experimental animals. Consequently, the mouse is a prime candidate for flight studies, with a selection of lymphocytic choriomeningitis, pneumonia, or mammary tumor viruses as candidate infectious agents. Although the appearance of clinical symptoms may be detectable remotely, recovery of the specimen would be required for confirmation of the causative agent of disease.

3i. Identification of the host factors that keep latent infections dormant and determination of the factors that may alter host defenses and thereby result in activation of a latent infection.

Except for the program recommended in (3h), which forms a part of this more inclusive recommendation, these studies should be performed in conventional ground-based laboratories.

3j. Investigation of the changes that may occur in microbial flora, particularly as they relate to selection of virulent agents, under conditions of long-term space-flight, utilizing experimental animals.

Each manned spaceflight and each recoverable unmanned spacecraft carrying experimental animals offers an opportunity to gather valuable data on the nature of the changes in microbial flora associated with the spacecraft environment.

Variations in the environment, on the other hand, are more susceptible to study in ground-based closed environment chambers, employing either man or animals for the studies, where varying the environment to extremes does not place a mission in jeopardy.

Because direct response of the microbial flora to weightlessness appears unlikely (p. 61) except through possible changes in the distribution of aerosols, it should be possible to determine the tendency toward selection of virulent agents in ground-based experiments. The influence of the stresses of actual spaceflight on the modification of the microbial flora of the host should be evaluated by comparison with ground control data to determine the need for spaceflight experimentation with experimental animals to evaluate selection of virulent strains. Model systems for such study might include Salmonella or Shigella carrier states or pathogenic fungi, and would employ recoverable unmanned spacecraft.

3k. Studies, both animal and human, of the effects of isolation on susceptibility to infection, particularly as related to the re-entry problem and microbial shock.

These studies should be performed in ground-based closed environment systems and would assume that the host defense mechanisms are not significantly changed as the result of weightlessness or changed periodically during prolonged spaceflight. If it becomes desirable to expand the objectives of this recommendation beyond that of the effect of isolation alone, animals from other experimental flight programs, both manned and unmanned, could be used to evaluate the likelihood of "microbial shock" upon re-entry into the earth environment.

III. CONCLUSIONS

The recommendations of the Space Science Board for research studies of infectious disease call for an extensive and multi-faceted program for the purpose of insuring the safety of space travelers which simultaneously addresses many of the problems which face the earth-bound medical community.

The spacecraft environment provides a unique mechanism to study the response of the animal organism to infectious agents under conditions of unusual and subtle physiological stress applied continuously and in a controlled manner, complementing the procedures employed in conventional laboratories.

The implementation of those recommendations will require considerable resourcefulness to maximize the scientific return while keeping cost within reason. Without more definitive descriptions of the individual experimental programs, particularly the number of flight experiments required, it is difficult to estimate the cost of such a program. The unabridged program would involve at least 10 long duration flights, manned and unmanned, for which the experiment-related costs could be expected to exceed $200 million dollars. It becomes apparent, therefore that priorities must be established and risk decisions must be made to enable concentration on the key problem areas.

An examination of the methods of implementation discussed in the previous section shows that much of the recommended program can be performed at relatively low cost in ground based laboratories. The type of experimentation and the experimental animals employed in the programs assigned to manned spacecraft are quite varied and complex, and generally require crew participation at a high level of technical proficiency. On the other hand, the experimental programs assigned to unmanned spacecraft fall essentially into two groups, one involving mice as the experimental animal, requiring large numbers for statistical validity, and the other requiring larger animals, perhaps four to six in number, involving samples of blood and perhaps feces or urine for analysis or preservation. Recovery of specimens for post-flight analysis and study is essential to each type of payload.

IV. RECOMMENDATIONS

The following recommendations are made with the purpose of establishing the steps required to define an acceptable program for the study of infectious diseases in spaceflight.

1. Define priorities for the research programs recommended by the Space Science Board, assessing the relative criticality of each, the risk involved if answers to the questions posed are not obtained prior to long duration space flight, the potential benefit to clinical medicine, and the order in which research programs should be undertaken.
2. Perform a comprehensive analysis of the flight experimental programs required to fulfill the recommendations of the Space Science Board, including at least the following.
    a) Review the experimental methods employed in conventional laboratories for research in infectious disease processes applicable to the problems to be studied in space flight experiments to define:
        1) Those techniques applicable to manned and unmanned spaceflight experimental programs.
        2) Species of experimental animals to be employed.
        3) Statistical rationale for the design of spaceflight experiments, including a definition of numbers of animals required per flight, number of flights and ground-based controls required.
        4) Investigators actively performing research in the specific areas of interest.

b) Define biological payloads and associated support hardware and instrumentation required to perform the experiments defined in (2a) on manned or unmanned spacecraft as appropriate.

c) Define the requirements and design concepts for experiment modules for the experiments defined in (2a) for use on manned or unmanned spacecraft as appropriate, which will provide the most practical, cost effective, and scientifically productive means of implementing the space flight experiments.

d) Estimate the total cost of implementing each experimental program, including the associated experiment development activity, to enable comparison with the benefit and risk assessments called for in (1).

V. LITERATURE CITED

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2. Enlow, D.L,. Experimental Investigation of the Phenomena Associated with Planetary Quarantine, American Association for Contamination Control Annual Technical Meeting, Anaheim, California, 1970.

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