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Chapter 9

PLANETARY QUARANTINE: PRINCIPLES, METHODS, AND PROBLEMS[1]

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FOUNDATIONS OF PLANETARY QUARANTINE

The Contamination Problem

Mankind has been fascinated since ancient times by the prospect of discovering and studying extraterrestrial life forms. Now that space exploration has become a reality, the detection of planetary life or its precursors has been established as a major objective of the planetary programs of both the United States of America and the Union of Soviet Socialist Republics.

The success of these space explorations, however, is threatened by the possibility that undesired life forms might accompany man and his vehicles from one planet to another and establish themselves with unforeseeable consequences. The introduction and proliferation of terrestrial life forms could destroy the once-in-forever opportunity to examine the planets in their pristine condition. Planetary quarantine is intended to safeguard this opportunity.

Perhaps Earth is the only planet in the solar system where life can exist. Should this prove to be so, some of our efforts will have been wasted. For the present, however, planetary quarantine appears to be the proper course of action for three reasons:

(1) Terrestrial microbial life carried to a planet by an automated or manned spacecraft may reproduce and spread on that body, confuse follow-on studies forever, and possibly mask or destroy life indigenous to that planet. The environment of the planet may be so changed that the planet's usefulness to mankind in future centuries will be seriously reduced.

(2) An automated spacecraft intended to detect biological life on a planet must not carry terrestrial microbial life, or the instruments may detect it rather than extraterrestrial life.

(3) The Earth may be adversely affected by organisms or materials brought back from another planet or from outer space.

Although these reasons for quarantine focus on microbial life forms as the primary contamination threat, because of their recognized ability to withstand environmental extremes and to reproduce singly, the interests of science in extraterrestrial life are not entirely confined to these forms. For example, the discovery of organic molecules that were the precursors or remnants of viable life would be of tremendous scientific significance. Accidental introduction of organic molecules or terrestrial life forms other than viable microorganisms could cause erroneous conclusions about life in the solar system. The quarantine program must address these possibilities.

The possibility of back-contamination of the

[1] The author expresses gratitude to V. I. Vashkov, N. V. Ramkova, T. V. Shcheglova, and B. B. Polyakov—Soviet authors of the review used in writing this chapter.

Earth by extraterrestrial life has applied so far only to manned flights to the Moon. The Lunar Receiving Laboratory (LRL), provided isolation for the returning Apollo astronauts and lunar samples. As information on martian environmental conditions accumulates, the importance of isolating and decontaminating returning Mars missions will become more evident. Meanwhile, plans must proceed on the assumption that quarantine against back-contamination will be necessary. The techniques required differ considerably from those necessary for prevention of outbound contamination by terrestrial organisms. One potential technique for unmanned missions involves remote inspection of returned samples while in Earth orbit—quarantine to be lifted and the samples brought to Earth only if tests for biological activity are negative. Another candidate technique involves encapsulation of the returned samples prior to Earth entry; a biologic barrier would then maintain quarantine during investigation of the samples on Earth.

The first significant discussions on planetary back-contamination were in Washington, D.C., in 1964, when the US National Academy of Sciences (NAS) held a conference on the potential hazards of back-contamination from the planets [17]. The recommendations of this conference, although weighted toward manned exploration of the Moon, have served as the basic guide in developing the philosophy and broad policies for the US program for control of back-contamination.

Development of International Planetary Quarantine Programs

Concern over planetary contamination led to international cooperation in a program of Planetary Quarantine (PQ). In 1957 the NAS in the US expressed deep concern that contamination by early space exploration could endanger scientific investigation of the planets. The International Council of Scientific Unions (ICSU) was urged to assist in evaluating contamination hazards and to encourage development of preventive measures. Subsequently, the ICSU formed an ad hoc committee, Contamination by Extraterrestrial Exploration (CETEX) [24], to review this issue. The committee recommended, in 1958, adoption of a code of conduct aimed at achieving a compromise between all-out lunar and planetary exploration on the one hand, and, on the other, providing maximum protection against terrestrial contamination of the Moon and planets.

At its meeting in 1959 [25], CETEX took the position that the contamination problem was an integral part of the duties of the ICSU Committee on Space Research (COSPAR), established the preceding October. As a result, COSPAR assumed responsibility for consideration of the contamination problem and has since issued a series of resolutions defining planetary quarantine objectives for launching nations.

Development of National Planetary Quarantine Programs

In response to these actions developing and clarifying the meaning of planetary quarantine, the USSR and US have undertaken planetary quarantine programs. This program is administered in the US by the National Aeronautics and Space Administration (NASA).

Those directing PQ operations in both nations strive to meet the objectives set by COSPAR by first establishing realistic PQ requirements and then monitoring spaceflight projects to verify that the requirements are satisfied. A primary quarantine objective was adopted by COSPAR in 1964 in a resolution recommending an upper bound of 1×10^{-3}—one chance in a thousand—for the probability that a given planet will be contaminated by a program of terrestrial space exploration consisting of a number of missions over a stated time period.

Acceptance of this definitive objective opened the way for development of quantitative PQ requirements for each spaceflight mission. In the procedure developed in the US, the PQ authority subdivides the upper bound of 1×10^{-3} on the basis of COSPAR's estimate of the number of flights capable of causing contamination and issues to each flight project an allocation limiting the probability that that mission will result in planetary contamination. The computation of these allocations recognizes the differing con-

tamination risks of the various types of missions such as landers, orbiters, and flybys. The allotment method employed generally favors orbiter and flyby missions at the expense of landers, since the former can frequently satisfy allocations through the use of highly reliable guidance components without resort to the expensive, though effective, decontamination procedures needed by landers. The allocations assigned to several US missions are in Table 1.

PLANETARY QUARANTINE METHODOLOGY

Planetary quarantine places constraints on planetary flight missions to minimize the probability of contamination of the planet and of the scientific experiments carried aboard the spacecraft. These constraints may affect the preparation of spacecraft and equipment for flight and the spacecraft trajectory. Since the spacecraft and equipment must be as reliable as possible to maximize the probability of mission success, care must be exercised in the selection of PQ techniques to assure that their application does not detract from the mission success.

This potential conflict of goals must be recognized both by those who establish PQ requirements and by those who select and implement contamination control measures. Effective administration and management of the PQ program must also include reasonable sensitivity to the technologic and budgetary constraints on flight projects. For these reasons, analytical and experimental studies are carried out to develop PQ techniques that have minimum impact on mission effectiveness and maximum cost effectiveness.

A major achievement of the United States PQ program has been the development of an analytical structure interrelating the various factors involved in the contamination estimation. Expanding upon a mathematical formulation of contamination probabilities first suggested by Sagan and Coleman [107], analytical models [53, 111, 119] now exist to facilitate the establishment of PQ requirements, measure their impact upon mission objectives, estimate potential contamination, determine decontamination needs, and select contamination control measures.

Models for Probability of Contamination

The manner in which the various factors interact, in determining the extent of contamination control needed, can be illustrated with the aid of a risk allocation model. Risk is defined in terms of the probability of occurrence of the various events that can lead to planetary contamination by each of the many distinct, potentially contaminating sources.

One such model, useful in determining the extent of decontamination needed during the assembly and test of a planetary lander, is based upon an analytical formulation by a working committee of COSPAR undertaken during its 1967 meeting in London [22]. It expresses the probability that at least one of the $N_i(o)$[2] organisms present on a spacecraft at launch contaminates the planet. It is a simplification of a more rigorous formulation and assumes that the product of the probability terms on the right side of the equation is much smaller than unity.

$$P(c)=\sum_i N_i(o)\cdot P(st)\cdot P(uv)\cdot P(sa)\cdot P(r)\cdot P(g) \quad (1)$$

The value of $P(c)$ is equal to the summation, over i distinct sources of microbial contamination, of the product of the number of microorganisms in each source and the probabilities of events occurring that effect contamination. In this equation [46]: $N_i(o)$ is the number of viable terrestrial organisms at launch. The events of concern in this example are:

[2] The microbial contamination in spacecraft, before terminal sterilization procedures are applied, is determined through assay and estimation procedures, which are described in the next section of this chapter under **Estimation of Microbial Contamination Levels.** In practice, the surface contamination is determined by assaying a large number of random surface samples and statistically treating the results to obtain a conservative value of the average number of microorganisms per unit surface area. This value is then applied to the total spacecraft surface area. Applying the same assay results to the portions of spacecraft surface areas which will be joined together determines the mated microbial burden. Buried contamination is generally determined by applying the value for the mean density of organisms—similarly developed as explained in the section, **Estimation of Microbial Contamination Levels**—to the total volume of nonmetallic spacecraft materials. Reference [31] provides examples of spacecraft surface assays and resulting total surface bioburdens for past US flight missions.

$P(st)$ – probability that a microorganism on the spacecraft survives the stresses of interplanetary space travel

$P(uv)$ – probability that a microorganism on the spacecraft survives the stresses of ultraviolet radiation in space

$P(sa)$ – probability that a microorganism that has survived the stresses of space travel will survive entry into the atmosphere of the target planet

$P(r)$ – probability that an organism on the spacecraft will be released in a viable state onto the planet's surface or into its atmosphere, given that it has survived space travel and atmospheric entry

$P(g)$ – probability that a viable organism, deposited at random on the planet's surface or in its atmosphere, will grow and proliferate

Each of the parameters on the right side of Equation (1) can be broken down into many subparameters. For example, the probability of growth, $P(g)$, can include the probabilities that the species of organism can grow on the planet, that a suitable growth environment exists on the planet, and that the organism will survive transfer from the spacecraft to a favorable environment. The final summation is a probability that can be compared directly with the probability-of-contamination allocation issued to that mission. This approach defines the limits of the probability of occurrence of undesirable events. A knowledge of this value and of expected fallout and indigenous levels then establishes the need for contamination control during spacecraft manufacture and preparation for launch.

TABLE 1.—*Probability of Contamination Allocations for US Missions* [1]

Mission	Probability of contamination
Mariner Venus 1	1×10^{-4}
Mariner Venus 2	1×10^{-4}
Mariner Mars 3	4.5×10^{-5}
Mariner Mars 4	4.5×10^{-5}
Mariner Venus 5	3×10^{-5}
Mariner Mars 6	3×10^{-5}
Mariner Mars 7	3×10^{-5}
Mariner Mars 8	7.1×10^{-5}
Mariner Mars 9	7.1×10^{-5}
Pioneer 10	6.4×10^{-5}
Mariner Venus Mercury for Venus	7×10^{-5}
Viking '75 (each launch)	1×10^{-4}

[1] Planetary Quarantine Status Board. NASA Headquarters, Planetary Quarantine Office of Space Science, Washington, D.C.

In this evaluation process, space survival terms must be determined, which is accomplished by the PQ authority with the aid of scientists and researchers in space microbiology and environment studies. Many special studies are initiated under the auspices of the PQ authority to gain insight into the pertinent phenomena and reduce the uncertainty in quantitative evaluations.

Studies of Space Survival Parameters

Studies have been conducted for the evaluation of space survival parameters by both the US and USSR. Experiments simulating deep space have proved that interplanetary environments are far less lethal to microorganisms than to other, more complex forms of life. However, some inactivation can be expected due to radiation, and to some extent from the stresses of temperature and vacuum.

The Jet Propulsion Laboratory (JPL) of the California Institute of Technology is investigating microbial survival in long-duration exposure (up to 180 days) to simulated spaceflight temperature and vacuum conditions. It appears [45] that the lethality attributable to these conditions is neither dramatic nor consistent, suggesting that reliance upon the combined effects of vacuum and temperature for significant microbial kill in deep space is not realistic. A slight initial reduction of the vegetative microbiologic population on the spacecraft can be expected, but vacuum and temperature stresses of deep space cannot be categorically regarded as sterilizing or decontaminating agents, particularly for spores.

Experimenters in the USSR as well as in the US have incubated various organisms under conditions simulating the environments of Mars, Venus, and the Moon. Under martian conditions of daily temperature alternation between −60° C

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and +26° C, atmospheric pressure of 7 mm Hg, and gas composition of 80% carbon dioxide and 20% nitrogen, several desert organisms [60] were able to grow at a relative humidity as low as 3.8%. The ability of such terrestrial life forms to exist on Mars probably depends on whether this minimal amount of moisture is available.

Outer space simulation tests in the USSR [59] showed that some microorganisms and enzymes are very resistant to vacuum pressures as low as 10^{-10} mm Hg. Furthermore, certain US tests [36, 95] indicate that vacuum may stabilize microorganisms. In interpreting the results of simulation experiments with vacuum, two factors must be considered. The first is that a relatively long time is involved in reaching a vacuum equivalent to space, especially if elevated temperatures are also required. The test organisms are therefore desiccated at a much slower rate than those located on the surface of a space probe during exit from the Earth's atmosphere. The second consideration, which is difficult to resolve satisfactorily, is the question of adequate controls. Even when moderate vacuums (10^{-3} mm Hg) are employed as controls, it is often difficult to establish the exact stage (application of the vacuum, extent of drying, or rehydration process) at which a cell either loses viability or is stabilized.

Ionizing space radiation other than that from solar flares and in trapped belts is not considered a significant inactivation stress; it is doubtful whether such radiation can be depended upon to destroy surface contaminants on space probes. For example, microorganisms inhabiting the water of nuclear reactors have adapted to millions of roentgens of radiation.

Ultraviolet light is a highly lethal agent in outer space. Table 2 lists the dosages necessary for 80 to 100% inactivation of unprotected microorganisms. These data are based upon US and USSR experimentation [21, 78, 109]. Despite a high degree of reflection, ultraviolet radiation is easily blocked by dust or other opaque material, such as an outer layer of microorganisms shielding underlying cells.

Experience in thermal effects of Earth reentry has been combined with postulated models of planetary atmospheres in estimating the probability that microbial contamination on landing vehicles will survive entry into the atmosphere. These analyses are generally performed by planetary spaceflight projects since many of the trajectory and engineering parameters involved are unique to a particular mission. The results, therefore, have limited applicability to other missions.

An analysis of survivability during entry into the atmosphere of Jupiter has recently been completed [116]. Extensive surface heating and ablation is predicted, based upon the high atmospheric density and vehicle trajectories that give high entry velocities.

TABLE 2.—*Resistance of Various Microorganisms to Ultraviolet Radiation* [34]

Microorganism	UV Dosage required for 80–100% inactivation in ergs/cm² × 10⁴		
	Ref [21]	Ref [78]	Ref [109]
Actinomyces sp	–	–	4.0–8.0
Aspergillus nidulans	–	–	54
Aspergillus niger	–	–	90–160
Bacillus megaterium	–	2.9	1.13
Bacillus megaterium	–	6.0	2.73
Bacillus pyocyaneum	–	4.4	–
Bacillus subtilis	7	–	6–7
Bacillus subtilis	12	–	12
Bacterium aertrycke	–	0.048	–
Escherichia coli	3	1.55	1–2.5
Micrococcus candicans	–	3.67	–
Micrococcus lysodeikticus	–	–	27–50
Micrococcus pyogenes aureus	–	–	6.0
Micrococcus radiodurans	–	–	80–160
Micrococcus sphaeroides	–	–	10
Oospora lactis	5	–	–
Penicillium digitatum	44	–	–
Pseudomonas aeruginosa	–	–	1.8–3.6
Pseudomonas fluorescens	–	–	3.0–3.5
Saccharomyces sp	–	14.7	–
Saccharomyces cerevisiae	6	6.5	–
Saccharomyces (diploids)	–	–	30
Saccharomyces (haploids)	–	–	8.4
Saccharomyces turbidans	–	9.0	–
Saccharomyces vini (diploids)	–	–	30
Salmonella typhimurium	–	–	1.9
Sarcina lutea	–	–	19.7
Serratia marcescens	–	0.7	1.8–4.0
Staphylococcus albus	–	–	1.84–3
Staphylococcus aureus	–	1.54	2.18–4.95
Streptococcus hemolyticus	–	–	2.16
Streptococcus lactis	–	–	6.15
Streptococcus ridans	–	–	2.0

Research has recently been completed on improved estimation of the probability that organisms buried in, or on, an arriving spacecraft will be released on a planet surface in a viable state. Of the several potential release mechanisms pertinent to landings on Mars, release resulting from material fracturing on impact and from aeolian erosion were assessed in a test program conducted by the Boeing Co. [82]. Inoculated pellets of spacecraft material were fractured in high-velocity impact tests and eroded by sand blasting. The tests have supported the specification of these parameters issued for use by US flight projects[3] (see Table 3).

TABLE 3.—*Values Specified for Probability of Microbial Release Parameters for Martian Missions under Conditions of Fracturing Impact and Aeolian Erosion* [30, 105]

Probability of release	Landing	Value
Surface contamination	Nominal	1 to 1×10^{-2}
Surface contamination	Non-nominal	0.5
Mated surface contamination	Nominal	10^{-3}
Mated surface contamination	Nominal	10^{-1}
Buried contamination	Nominal	10^{-4}
Buried contamination	Non-nominal	10^{-4}

The estimation of $P(g)$, the probability that a terrestrial microorganism reaching a given planet will grow and proliferate, has received considerable attention. Laboratory tests of the ability of terrestrial organisms to grow in simulated martian environment were carried out with inconclusive results due to lack of knowledge of the availability of water in the microenvironments of the atmosphere. The Space Science Board of the NAS reviewed the evaluation of $P(g)$ for Mars in July 1970 and recommended using a value of 1×10^{-4}, admitting, however, that this estimate reflected incomplete knowledge of the martian environment, and would be subject to reevaluation when further data became available. Data from US and USSR missions to Mars may support such a reevaluation.

[3] *Planetary Quarantine Specification Sheets.* Sept. 28, 1972, Revision 1, Nov. 1972. Issued by NASA Headquarters, Planetary Quarantine Officer.

These few examples illustrate how a laboratory-based research program has eliminated information gaps and helped develop quantitative space survival probabilities that can be technically substantiated and effectively utilized in establishing valid contamination control procedures.

STANDARDS AND GUIDELINES

In determining the need for contamination reduction and control procedures, microbial contamination levels must be anticipated. The effect of spacecraft assembly and test operations upon these levels must also be estimated.

The PQ authority assists, on the basis of laboratory research, in the estimation of these parameters and guides flight project groups by issuing contamination control technology information with guidelines for its application.

Estimation of Microbial Contamination Levels

Microorganisms can be enumerated through assay, in the case of accessible surface contamination, or by estimation, for those levels which cannot be directly sampled without damaging the spacecraft.

Accessible contamination. The accurate enumeration of microorganisms on surfaces requires recognition and consideration of a number of factors. Surfaces associated with spacecraft are composed of a wide variety of materials with many surface finishes. In addition, some of the materials contain substances that inhibit the growth of microorganisms. Consequently, assay procedures for space hardware have been designed to separate microbial contamination from surfaces for subsequent culturing.

Microorganisms that have been present on surfaces for long periods, or that have survived decontamination treatments, tend to adhere quite tenaciously. The consequent difficulty of removal can markedly affect the accuracy of the assay. In addition, microorganisms in nature usually do not occur as single cells but rather in clumps and aggregates made up of many cells. Standard techniques for the microbiologic sampling of surfaces [32, 100] such as the rinse, swab-rinse, and direct contact plate [48], do not dis-

tinguish between individual cells and aggregates of cells and hence can lead to enumeration inaccuracies. Although the rinse and swab-rinse tests do allow for some breaking up of clumps by manual or mechanical shaking, there is variation in detection counts. Recovery efficiencies vary not only according to size, composition, and finish of the surfaces, but also among personnel performing the assays.

New techniques have been developed not only to reduce this variability, but also to increase recovery of microorganisms from space hardware. One technique, the vacuum probe, resulted from a study initiated to develop a surface sampler that could be used on large surface areas [76, 103].

Puleo et al [100] used an ultrasonic vacuum probe to effectively recover microorganisms from a variety of surfaces, even when bacterial spores were "heat fixed" to the test surfaces. Ultrasonic energy broke up clumps of microbial cells into smaller aggregates and single cells. Both factors, removal and declumping, markedly improved the accuracy of the assay. Subsequent studies [99] showed that ultrasonic baths produced the same recovery efficiencies as the ultrasonic probe. Also, the ultrasonic baths were easy to use and permitted processing more samples in a given period. This procedure works equally well for spores and vegetative microorganisms and does not produce any detectable lethal effects on microorganisms. Consequently, ultrasonic energy is employed in assaying microbial surface contamination, whether on stainless steel fallout strips, piece parts, swabs from the surfaces of space hardware or filters, or probe tips of vacuum probes. Vacuum probes and ultrasonic collectors cannot be used on spacecraft, however, since the ultrasonic energy can damage sensitive parts. As a result, there is almost complete reliance on the swab-rinse technique in detecting surface contamination levels. Ultrasonic energy can be applied as soon as the sample has been recovered from the spacecraft material surface. A second innovative collection technique, capable of removing a large sample of surface contamination, has been developed by the Phoenix Laboratories of the US Public Health Service, Center for Disease Control. The sample is collected by wiping the entire surface of interest with a sterile, rayon cloth (commercially available in the US). Microorganisms collected by the cloth are recovered by sonication in a rinse solution which is then assayed. This wipe-rinse technique exhibits a sampling efficiency comparable to that of other techniques but has the marked advantage of recovery from larger surfaces and consequently produces more valid results [33].

Inaccessible contamination. Contamination located between joints (mated contamination) or encapsulated within material is generally inaccessible for direct assay; its levels are estimated by methods and guidelines established through experimentation and experience. Mated contamination is accessible on the surfaces of the parts prior to hardware assembly, and can be assayed at that time. The usual approach to its enumeration is to establish, by assay, the surface contamination density existing at the time of assembly, and to use this value in conjunction with the extent of mated surface areas to develop a conservative estimate for the total mated surface contamination level. In this approach the assumption is made that there is no growth or die-off once the parts have been permanently mated.

Viable organisms encapsulated within solid materials can be released to a planet surface if the solid should be fractured, eroded, or deteriorate. In an effort to provide a basis for estimation of microorganisms contained within spacecraft materials, several research groups have attempted quantitative recovery of viable microorganisms from solid materials. US investigators showed in 1960 [87] that certain types of electronic piece parts contained viable microorganisms internally. As a result of this work, JPL investigators and their subcontractors in the Dynamic Science Corp. initiated studies to define both microbiologic and engineering problems of interior contamination assay [76, 103]. Subsequently, similar studies were initiated by workers in the Phoenix Laboratories, US Public Health Service (USPHS), Center for Disease Control [101], the USPHS in Cincinnati [4, 5, 6], and at the University of Minnesota [43]. The pertinent results of these studies can be summarized in the following paragraphs.

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In conducting assays of encapsulated microorganisms, the objective is to reduce solid material to a size small enough to ensure release of embedded microorganisms without harming them. If microorganisms are killed, the extent of kill should be consistent from test to test.

No single assay system is suitable for measuring the internal contamination of all potentially contaminated materials. Hard materials can usually be ground or pulverized, but the rate of kill for embedded microorganisms may be high.

Some polymers can be dissolved by nontoxic solvents to free embedded microorganisms which can then be plated out and counted [49]. Relatively soft materials which do not lend themselves easily to pulverization at ambient temperatures can be treated with liquid nitrogen, which makes them brittle enough for pulverization. However, this problem is far from solved.

Some materials contain toxic substances which must be chemically neutralized or physically removed from the microenvironment prior to culturing.

Solidification, polymerization, and storage of solids can contribute to a significant die-off of embedded test microorganisms when model systems are used to evaluate various recovery techniques.

Recent work in the US conducted jointly by the USPHS and Exotech Systems, Inc., resulted in an estimation technique for buried bioburden based upon an analytical model that accounts for the uncertainties of assay techniques [73]. The model was exercised using all known data obtained by laboratory fracturing, grinding, dissolving, and disassembling, as appropriate, various samples of solid materials and spacecraft piece parts. The results led to a specification conservatively stated as 130 spores/cc as a mean density of organisms in nonmetallic spacecraft materials.

This conservative specification can be used in estimating the contamination encapsulated in materials produced in the US as they are received for fabrication and assembly. Such information can eliminate the need for extensive bioassay by flight projects. As further aids to flight projects, standards and advisory procedures have been issued by the PQ authority which include selection of a standard test microorganism, decontamination and sterilization methods, standards for microbiologic assay, and values for microbial death rates. These standards are based upon experimental investigations sponsored, in the main, by the PQ authorities of the USSR and the US.

The *Bacillus subtilis* var. *niger* spore has been selected in the US as a representative organism for testing the effectiveness of decontamination or sterilization procedures used in a planetary flight program [80]. Extensive knowledge exists about this microorganism and standardized cultures are readily available from the Phoenix Laboratories, Center for Disease Control. Its dry-heat inactivation characteristics are well-known from extensive studies and are being compared with those of naturally occurring contamination experienced in spacecraft fabrication and testing in substantiating sterilization processes [120].

Spore inactivation in dry-heat sterilization is specified in terms of a *D*-value, the time required to reduce the mean viable microbial population by a factor of 10. An important question in using a *D*-value to describe the die-off behavior of microorganisms is whether or not inactivation kinetics are exponential, i.e., linear on a semilogarithmic plot, or instead possess either a shoulder or a tailing effect. The presence of a shoulder suggests the need for additional time to reach a specified level of decontamination and a tailing effect indicates that a portion of the population will survive a given treatment, independent of the duration of exposure. Either effect may negate much of the usefulness of the *D*-value concept in establishing sterility requirements. Despite the current uncertainty of the validity of the exponential approximation, the *D*-value concept has been found operationally useful in the design, implementation, and verification of heat sterilization measures. Further research to detect any tailing effect in naturally occurring populations is underway in the US.

The *D*-value is a function of the accessibility of the microbial population and the temperature and moisture content of the surroundings. In

practice, D-values are specified at 125° C with a maximum allowable moisture content. A scaling factor, defined as a Z-value, permits extrapolation of D-values to other sterilizing temperatures. Table 4 presents dry-heat sterilization specifications currently in use in the US.

TABLE 4.—*Dry Heat Specifications*

Temperature	100°C min.
Max. RH	25% at 0°C and 76 mm Hg
D-Values	
Buried burden	5 h
Mated surface burden	1 h
Exposed surface burden	1/2 h
Z-Value	21°C
Temperature scaling function	$D_T = D_{125} \cdot 10^{\frac{125-T}{Z}}$ where D_T is D-value at temperature T in °C

Other standards have been issued to cover contamination control methods. Both heat and ethylene oxide are acceptable decontamination techniques for US spaceflight programs. The USSR employs these and other techniques (described in a later section). Guidelines resulting from experimentation describe the use of barriers to limit microbial accumulation in and on spacecraft. A description of the methods for reducing the number of microbes in spacecraft assembly areas is given in the next section, CONTAMINATION CONTROL TECHNIQUES.

Contamination Source Analysis

A probability-of-contamination analysis is performed by flight projects to determine the need for contamination control and to assist in selection of proper control measures. Determining the probability of contaminating a planet requires: (1) identifying all possible contamination sources associated with a mission; (2) determining the initial contamination levels associated with each source; (3) determining the contamination level at time of launch; (4) determining whether this level—or some fraction of it—can physically reach the planet; and, (5) determining what fraction of the contamination level which does reach the planet will survive the environmental stresses encountered prior to reaching the planet.

An illustration of contamination source identification [26, 80] for a planetary lander project is in Figure 1. The key points of this contamination analysis by the JPL are:

(1) All aspects of the flight program, including the interactions of flight hardware with the interplanetary environment, are examined to identify possible sources of contamination.
(2) Sources of contamination are examined individually to yield an adequate understanding of the processes through which they occur.
(3) Where possible, mathematical models are formulated which characterize the probability of contamination. The models are based on standard probabilistic techniques, and the limitations inherent in their formulation are described.

The major possible sources of contamination [52] for an orbiting mission are shown in Figure 2. Hall [47] pointed out that the quarantine constraints for accidental impact of the nonsterilized spacecraft can have a dominant effect on the trajectory biasing requirements and the orbit altitudes; consequently, close scrutiny of acceptable risks for flyby and orbiter missions is warranted.

The initial burden of viable organisms on the vehicle is determined by extrapolation from microbial assay and can be expressed in any of several ways. It can be stated as an average of the several measurements of the bioburden. A more conservative approach is to state it as an upper or maximum value of several measurements of the microbial contamination level. A system model, used by Ingram [61], treats this number as several ranges of values, each with an associated probability—i.e., a histogram. Implementation of Ingram's model, however, is complex, requiring a knowledge of every contaminating event during assembly and of the associated environments. A stochastic model has been developed which avoids these difficulties [106]; this approach is being considered for use

574-269 O - 76 - 28

by the US Viking 1975 project. Methods have been developed for systematically dealing with histogram data in probabilistic computations with parameters of variable uncertainty [51, 110].

Documentation

Flight project groups are required to document the results of their analyses and the measures used for contamination control. US projects prepare planning documents (including a PQ plan, microbiologic assay and monitoring plan, and sterilization plan) and analysis reports. Planning documents are submitted to the PQ authority for review to ensure adequate attention to decontamination needs and measures. Analysis reports document the analytical development of decontamination requirements and quantify the constraints, if any, to be applied during spacecraft fabrication.

Prior to approval for launch, a report is submitted to the PQ authority describing both the compliance measures undertaken by the flight project, and their effectiveness in limiting the microbial burden during manufacture. Based upon the acceptability of these data as well as an independent verification conducted by the PQ authority, an approval for launch is granted.

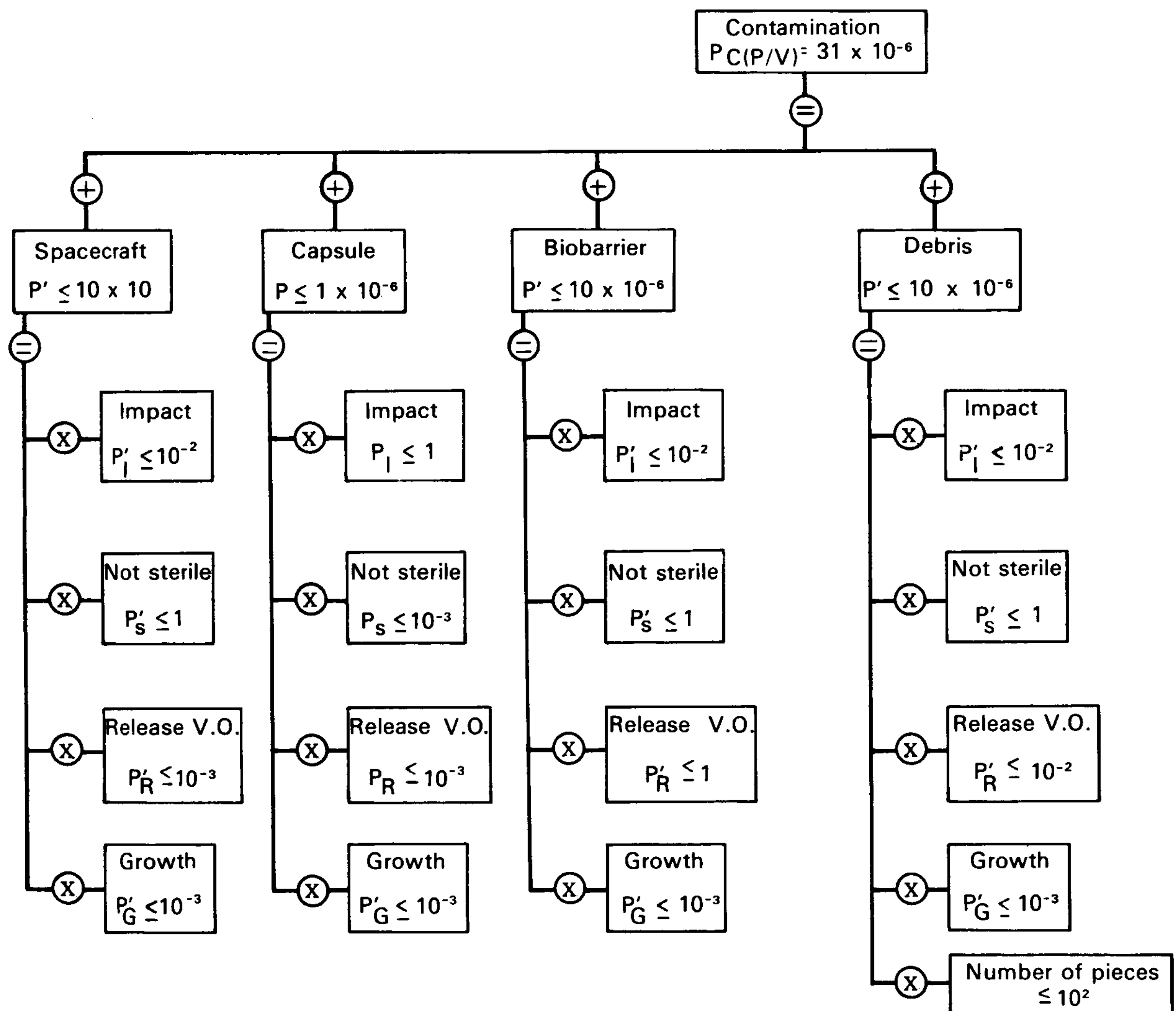

FIGURE 1.—Contamination source identification for planetary lander mission.

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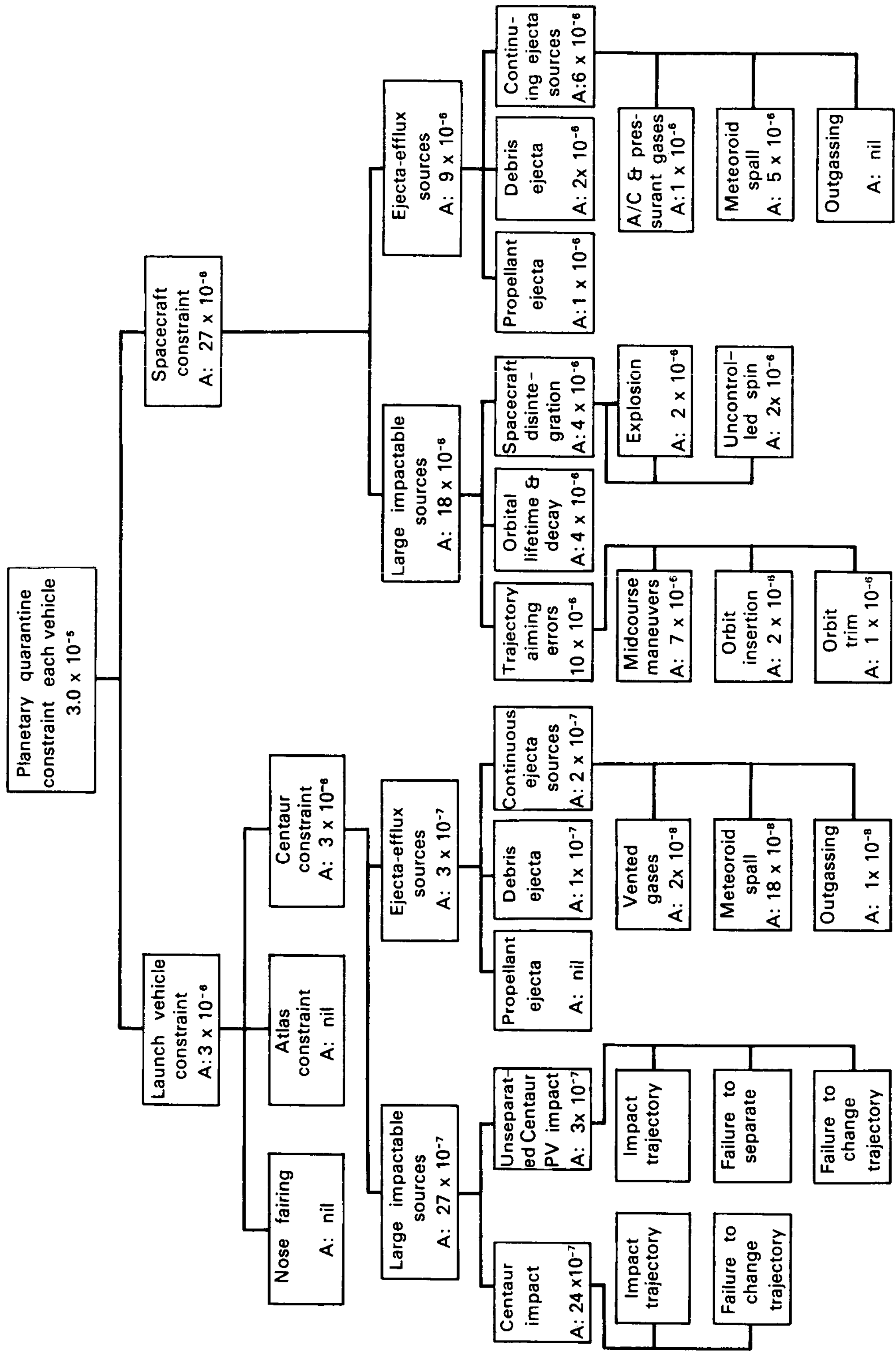

FIGURE 2.—Mariner Mars 1971 planetary quarantine model.

Launching nations are urged to submit to COSPAR a report stating the degree to which spaceflight missions have satisfied quarantine requirements. The data are summarized to aggregate the estimated probability that the total space exploration programs of all nations have contaminated each planet. Current results are examined by the COSPAR Panel on Planetary Quarantine at each annual meeting to assess the progress of the total Planetary Quarantine program and to determine the need for policy revisions. Complete international cooperation in this matter has not yet been attained by COSPAR, but full participation should result when all launching nations accept the importance of adequate PQ measures.

CONTAMINATION CONTROL TECHNIQUES

The value to successful quarantine of measures such as a constituted PQ authority, an awareness of the problem to be solved, and development of the quantitative requirements and implementation guidelines have been illustrated in preceding sections. However, it is the adequacy of the measures taken by the spaceflight group to control the level of contamination of space vehicles and to produce the operational reliability needed to minimize the chances of a contaminating accident that assures that the quarantine goals are met. On the basis of a contamination analysis, contamination control needs can be determined and related to measured bioburdens at key times during the assembly process. To meet these needs, control measures can be selected to be applied during assembly stages. In similar fashion, operational reliability goals can be quantified and used to specify design quality, workmanship standards, and reliability assurance levels to be achieved when the number of microorganisms does not exceed the level established as the highest acceptable limit. Obviously, the most stable and desirable level of control from a microbiologic viewpoint would be sterility—the absence of viable microorganisms. Although sterility is the objective of spacecraft decontamination measures, it is always a matter of probability whether this absolute level is achieved.

Because of the possible adverse effects of stringent sterilization techniques on system performance it is undesirable to apply treatments to realize extreme probability of sterility. The probability of contamination achieved in recent US missions is illustrated in Table 5, which summarizes the estimated surface bioburden at the time of launch. Comparison with the surface contamination levels of products manufactured without biologic control indicates that a very low level of contamination has been achieved.

Preventive and corrective tactics are used in combination for contamination control: techniques for limiting the introduction of contamination and for reducing extant contamination levels of facilities, personnel, and hardware. Techniques have been adapted from medicine, food processing, environmental control, and other areas. Many have been further developed, and some new techniques have been devised. Undoubtedly, the advances in contamination control made for Planetary Quarantine will be used in turn by those areas from which the technology was adapted.

Contamination Prevention

Contamination prevention involves consideration of potential sources of contamination and the use of barriers to shield the spacecraft from these sources [74].

Biologic barriers. The objective of a biologic barrier is to keep the number of microorganisms within an enclosure at an acceptably low level. This can be accomplished by using an air shield as in a clean room or a solid microbial barrier.

Clean rooms reduce or eliminate microbiologic contaminants, at a site or on equipment, thus increasing the probability that subsequent decontamination will be successful. Functional reliability is also improved because of increased chemical and particulate cleanliness [79]. The effectiveness of a clean room in limiting the microbial level accumulated on the surfaces of spacecraft material depends upon the air-handling system employed and upon operational procedures. The control of airborne contamination is specified in terms of the maximum number of particles permitted in the air stream;

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e.g., a Class 100 clean room has less than 3.5×10^3 particles/m^3 (100 particles/cu ft). This low level is usually achieved by a vertical laminar flow, as opposed to turbulent flow air systems. Laminar-flow clean rooms and work areas successfully hold intramural microbial contamination to a very low level and drastically reduce soil-type microorganisms, such as bacterial and mold spores, from the extant microorganism population.

A vertical laminar-flow clean room usually has a full ceiling filter bank of high efficiency particulate air (HEPA) filters. The retention efficiency for typical HEPA filters often exceeds 99.6% for particles of 0.3 μm and larger. Exhaust air exits through a grated floor. The airflow is high volume and downward throughout the entire cross section of the room. A laminar horizontal, or crossflow clean room is somewhat less effective downstream from any source of contamination,

TABLE 5.—*Status of Probability of Planetary Contamination* [1]

Mars missions	Launch date	Type mission	Weight kgm	Probability of planetary contamination			Sterilized	Surface microbial spore load	Mission results
				Prelaunch allocation	Postflight estimate	Cumulative probability			
Mariner 3	11/5/64	Flyby	261	4.5×10^{-5}	NIL	NIL	No	2×10^5	Unsuccessful-failed to jettison
Mariner 4	11/28/64	Flyby	261	4.5×10^{-5}	NIL	NIL	No	2×10^5	Passed Mars at 9850 km 7/14/65
Mariner 6	2/24/69	Flyby	380	3×10^{-5}	approx. 3.2×10^{-8}	approx. 3.2×10^{-8}	No	2×10^4	Passed Mars at 3400 km 7/31/69
Mariner 7	3/27/69	Flyby	380	3×10^{-5}	approx. 3.2×10^{-8}	approx. 6.4×10^{-8}	No	3×10^5	Passed Mars at 3540 km 8/5/69
Mariner 8	5/8/71	Orbiter	990	7.1×10^{-5}	NIL	approx. 6.4×10^{-8}	No	1×10^4	Failed to achieve Earth orbit
Mariner 9	5/30/71	Orbiter	990	7.1×10^{-5}	3.9×10^{-5}	approx. 3.9×10^{-5}	No	1×10^3	Achieved Mars orbit 10/14/71
Venus missions									
Mariner 1	7/22/62	Flyby	202	1×10^{-4}	NIL	NIL	No	2×10^5	Unsuccessful—mission abort at launch by safety officer
Mariner 2	8/27/62	Flyby	203	1×10^{-4}	3×10^{-17}		No	5×10^5	Flyby passed Venus at 34826 km 12/14/62
Mariner 5	6/14/67	Flyby	245	3×10^{-5}	3×10^{-17}	NIL	No	assayed 1×10^5	Flyby at 3200 km 10/19/67

[1] Exotech Systems, Inc. *Contamination Logs for Mars and Venus.* Washington, D.C., Exotech Systems, 1971. (Rep. Contr. NASw-2062)

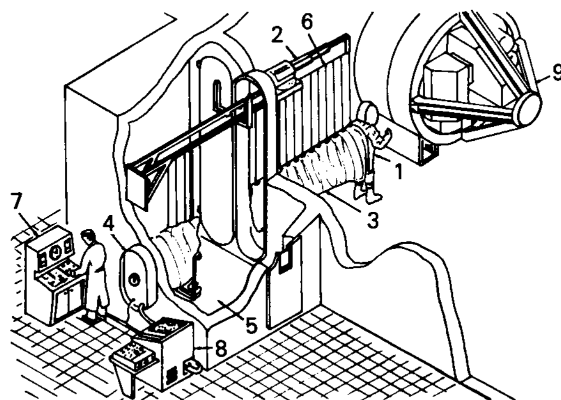
such as workers. There are also portable clean rooms—vertical laminar airflow units with plastic curtains for side walls, blowers, a ceiling HEPA filter bank, and support legs on casters to facilitate movement.

The JPL developed an Experimental Assembly and Sterilization Laboratory (EASL) [65, 75, 84] having an extremely low level of airborne microbial contamination in the bioassay room and laminar downflow work areas [74]. JPL later enlarged and improved the EASL and constructed a Sterilization Assembly and Development Laboratory (SADL) [102], a complete spacecraft assembly facility incorporating (1) a 130-m^2 laminar downflow room with a 10-m high bay; (2) an operational support equipment area; (3) a 75-cm ethylene oxide decontamination chamber; (4) a 75-cm dry-heat sterilization chamber, and (5) all equipment needed to conduct microbiologic assays.

Microbiologic barriers, from cabinet size to very large installations, prevent passage of microorganisms into or out of the barrier [88, 89]. In planetary quarantine, protection of the equipment or material inside the barrier is usually of primary interest, but occasionally a barrier that works in both directions is needed. The absolute barrier cabinets used in the US Lunar Receiving Laboratory prevented escape of potentially harmful lunar material during quarantine, and also prevented contamination of lunar samples by terrestrial microorganisms. Absolute barriers permit no interchange of protected and unprotected environments. Isolation is accomplished by placing material or work within a gas-tight enclosure, through the walls of which operations are conducted by means of flexible gloves or remotely operated instruments. In fact, such enclosures can be evaluated for microbiologic integrity with a gas leak detector.

The partial barrier principle uses enclosures, rooms, or cabinets that are not gastight and depend on air pressure differentials through working openings. Inlet or outlet air (or both) may be filtered. Pressurization of such barrier systems depends on whether work or personnel is to be protected. When work within the barrier is to be protected from outside contamination, the enclosure is maintained at positive pressure. Negative pressure is used in the enclosure to inhibit escape of microorganisms.

A Bio-Isolator Suit System (BISS), part of the assembly sterilizer facility designed in the US for the isolation of sterile spacecraft, has an outer suit, and an inner suit which is sealed to a flexible pleated tunnel large enough to permit operator access and egress (see Fig. 3) [38, 136]. The BISS is complete with life-support and monitoring subsystems. All human operations are topologically and biologically isolated by the suit and tunnel from the spacecraft after the beginning of the sterilization operations.



1. BISS outer suit
2. Boom
3. Tunnel
4. Hatch
5. Antechamber
6. Stringers
7. Director's console
8. Life-support console
9. Spacecraft

FIGURE 3.—Bioisolator suit system and assembly/sterilizer interface concept [74].

A comprehensive review of absolute barrier devices for personnel protection has been made by Gremillion [44], whose description of barriers includes incubators, refrigerators, centrifuges and balances, and covers disinfectant dunk baths and autoclaves for the entrance and exit of materials. Similar cabinets have been described by Blickman and Lanahan [13], and McDade [74] discussed barriers for aerospace application.

Flexible plastic barriers for the absolute containment of germ-free animals are largely the result of development work by Trexler [121]. A negative-pressure safety hood made of flexible

plastic film was developed by Phillips et al [91]. Subsequently, rigid plastic absolute barriers were also developed [90]. The types of barriers or isolators for germ-free animal experimentation have been summarized by Trexler [122, 123].

Preventing contamination by personnel. The primary source of microorganisms in spacecraft assembly is the personnel associated with the fabrication processes. Human skin surfaces provide a fertile site for the survival and growth of microorganisms [29, 72]. Bacterial studies have shown that microorganisms may be found on the skin surface, throughout the stratum corneum, and in the ducts of most excretory glands.

The removal of microorganisms from skin surfaces, or their sterilization, has been attempted many times by a variety of techniques. Surgeons rely upon the scrub-rinse technique generally employing hexochlorophene to reduce the number of organisms on the skin. Fingertips, of special interest, are the body surfaces most frequently in contact with spaceflight hardware. While the scrub-rinse technique tends to remove considerable numbers of skin microorganisms, sterility cannot be obtained [132].

Germicidal soaps help to suppress the bacterial skin flora for a time (as shown by Ulrich [125]) although the bacterial skin numbers eventually return to the level that is normal for the specific individual. It is quite probable that bacterial populations lying deep in the skin are well protected from biocidal concentrations of germicide, as well as against mechanical removal. Most probably, too, these organisms constitute the source of the eventual return to the normal bacterial skin level for that individual. Fortunately for the purposes of planetary quarantine, few, if any, spores or sporeforming bacteria are disseminated by the human body, although a few survive passage through the digestive tract and can be released to the occupied environment.

A noninjurious method of sterilizing skin is not known at present. Since bacteria are constantly shed or removed from the skin, a mechanical barrier such as surgical gloves, combined with the use of germicidal soaps, appears to be the best method of reducing or preventing microbial transfer from skin surfaces to spaceflight hardware.

Decontamination Techniques

Many techniques for reducing microbial levels on spacecraft and their components have been investigated. None is ideal, but several are presently in use and others show promise. Tests show that a higher degree of sterilization can be achieved when these techniques are used on a smooth surface. Contaminant survival becomes significant as surface roughness increases. The techniques described in this section include: direct contact disinfectant cleaning using solvents and decontaminants, remote surface decontamination with chemicals or radiation, and heat sterilization with and without radiation.

Disinfectant cleaning. Direct contact disinfectant cleaning involves washing the exposed surfaces of spacecraft materials with disinfectants such as ethyl alcohol, isopropyl alcohol, formaldehyde in methanol, paracetic acid, and hydrogen peroxide. NASA's AIMP Project [70] employed a disinfectant cleaning program including brush-washing electronic modules with ethyl alcohol, rinsing electronic components in 85–95% isopropyl alcohol baths, sponging surfaces with cotton swabs and wipers saturated with isopropyl alcohol. It was estimated that at launch a microbial surface level of 1.5×10^4 organisms was achieved.

Similar techniques have been used to limit the microbial surface contamination levels on the US Mariner program. Polypropylene pads saturated with a solvent selected for compatibility with the material being cleaned were employed in the Mariner Mars '71 program. Residues were removed by rubbing the surfaces with sterile flannel. On delicate parts, such as the solar panels, cotton-tipped toothpicks were used to minimize damage to the delicate electrical connection network.

Hydrogen peroxide, used in industrial applications for its biocidal properties, is suitable for use on some spacecraft materials. A concentration of 3 to 5% is effective for microorganisms in the vegetative state, 3 to 10% for spores (depending upon type), and a concentration of 1 to 5% will kill viruses [129]. A disadvantage of hydrogen peroxide is a high surface tension, 73 dyn/cm. This can be reduced by adding surfactants, such as 0.5% sulfanol, and achieve

cleaning through more complete coating on the surfaces. The addition of anionic detergents can reduce the surface tension of the solution to as low as 28.8 dyn/cm. Treatment of surfaces with such a mixture will have a lethal effect on vegetative and sporogenous forms of microorganisms, according to Shumayeva [113]. Immersion in a 6% solution can decontaminate surfaces infected with spores of malignant anthrax; and by increasing the temperature of the solution to 50° C, the concentration of the hydrogen peroxide can be reduced by a factor of two without decreasing the effectiveness.

Surface sterilization. The techniques refer to the use of chemicals or radiation without direct contact with the surface. Decontaminants used include gases (ethylene oxide, methyl bromide and formaldehyde) and radiation (laser beams, gaseous plasma, ultraviolet and ionizing radiation).

Ethylene oxide (ETO) has been used for many years in the pharmaceutical and food processing industries as a microbial decontaminant. Its effectiveness is a function of time, temperature, concentration, and relative humidity [115]. As a surface decontaminant for reducing microbial populations on spacecraft [66, 85], several factors must be considered. ETO is both toxic and hazardous. A small percentage (3%) in air will support combustion, and if ignited in a closed space will explode. However, a noncombustible mixture can be made by adding an inert substance. ETO mixed with carbon dioxide or various fluorinated hydrocarbons is available in the US. These mixtures do not support combustion with air in any proportion. Adverse effects of ETO include proneness to corrosion of materials; polymerization caused by catalytic properties of some materials; chemical reaction with some materials [37, 64, 67]; and parameter drift of some electronic devices [11, 14]. Such effects are overcome by proper handling and precautions.

Space scientists in the USSR currently consider a mixture of 40% ETO and 60% methyl bromide (referred to as OB mixture) the most suitable means of surface decontamination of space hardware. It is reported to be five times more effective against *Staphylococcus aureus* and two-and-a-half times more effective against spores of *B. mesentericus* than a mixture of 12% ETO and 88% Freon (referred to as cryoxide). The combination of ETO and methyl bromide exceeds cryoxide in bactericidal properties because of the bactericidal capacity of both components. USSR research indicates that the OB mixture does not harm materials being sterilized [83], but this remains an open question in the US.

Methyl bromide by itself has several valuable attributes. It is widely used as a refrigerant and in fire extinguishers, and is an excellent fumigant for stored grain, seeds, and nursery stocks. It is not as effective a sterilant as ETO, but adds a penetrating effect so that the combination of ETO and methyl bromide kills microorganisms more rapidly than either constituent alone, according to Soviet reports [127].

Recent research using formaldehyde gas generated from paraformaldehyde [86] suggests that it may have some use in the sterilization of spacecraft components. Formaldehyde as a gaseous sterilizing agent is advantageous in that the space to be sterilized need not be tightly enclosed nor hermetically sealed, a condition often impossible to achieve when large volumes are to be treated. Formaldehyde gas has a slow rate of penetration, a significant feature when residual action is required. The absorbed surface film of the polymer will continue to release formaldehyde gas slowly, often for several days after the sterilization cycle has been completed. If sterilization is carried out in a vacuum chamber, penetration can be accelerated. The principal disadvantages using formaldehyde as a surface sterilant for spacecraft are its interaction with some commonly used materials and its potential as an organic contaminant of life-detection experiments.

There has been renewed interest in formaldehyde sterilization recently because of the availability of a highly purified form of paraformaldehyde which apparently increases ease of production of the gas and reduces problems of residuals. Heating of this polymer results in the release of pure formaldehyde gas with little or no waste. An odorless formaldehyde-based sporicide is under development by Sandia Laboratories.[4]

[4] Presentation by R. E. Trujillo at the Spacecraft Sterilization Technology Seminar, San Francisco, July 1972. (NASA contract W-12-853)

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Both gel and liquid forms have been produced which have bactericidal effects similar to simple formaldehyde and accelerated effects with modest heating.

Researchers at MIT [97] have found that bacteria can be destroyed by irradiation with laser light. Heat-resistant spores (*Bacillus subtilis*) became entirely inactive within a hundredth of a second in a 50-W, unfocused CO_2 laser beam. The laser beam method does not disturb the substrate materials used (copper, aluminum, glass, and paper). This quality would make it possible to sterilize the surfaces of materials that are quite sensitive to heat—materials, in addition to those of spacecraft, include medical and surgical materials, foods, metals, paper, and probably even the surfaces of wounds during operations, as well as the air in the operating room. A wide range of wavelengths (from 2 to 10.6 μm) has been found effective in sterilizing spores. The high reflective energy produces extensive kill, mitigating possible structural shielding effects.

Gaseous discharge or plasma sterilization has been investigated as a means for complete elimination of contamination from medical products and foods [56]. The Boeing Co.[5] has tested the effectiveness of radio-frequency-generated oxygen plasmas on inoculated strips. A surface cleaning effect has been observed with plasma temperatures of 50° C. Sterilization appears to be complete without material degradation. The plasma is an extremely rich source of ultraviolet light, which may be the actual sterilizing agent.

Both plasma and laser beam sterilization are surface treatments with limited penetration ability. The applicability of these techniques is questionable to sterilization of surface films of thickness greater than 1 μm and of contamination resident in deep pores or sharp recesses.

Ionizing radiation [58] in the form of gamma rays, high-energy electrons and protons, is a decontaminant. Imshenetskiy and Abyzov reported in 1972 that in many cases where heating could damage electrical apparatus, the use of gamma rays is becoming increasingly popular in the USSR [56]. According to the data of Jaffe [62, 63] the γ-radiation dose required for sterilization of materials and apparatus on a spacecraft is 10 million rads. However, Astafayeva et al [8] feel that these doses are much too high. Their data indicate that sterilization of radio parts, even those containing spores of *B. mesentericus*, which are relatively resistant to the action of gamma rays, can be accomplished with a dose of 2.5 million rads. The same dose proved effective for sterilization of porous rubber and certain liquids that were considerably contaminated by a test culture.

Tests conducted in the USSR [126] of radiation-sterilized materials and radio parts after storage for various durations (from 1 day to 3 months) showed that physical and chemical properties of the materials and the electrical parameters of the devices tested remained unchanged. These studies indicated that the use of radiation sterilization is suitable in many cases for sterilizing spacecraft but would necessitate special attention to parts selection and design for radiation resistance.

High-energy electrons and protons, while not possessing the penetrating power of γ-rays, can sterilize surface contamination. Through reflection and scattering, they can also be effective on hidden surfaces. Both electron and proton beams can be focused in concentrated patterns, thereby speeding the decontamination process. Tests [3] underway at the JPL to investigate the lethal properties of the trapped radiation belts of Jupiter, should provide further data on the effectiveness of electron and proton beam sterilization.

Considerable information exists on radiation damage to materials similar to those used on spacecraft. A large segment of this information concerns nuclear reactor radiations (mixed neutron-gamma fields) which are believed unsuitable for spacecraft sterilization application since they can produce radioactivity in the irradiated material [12, 35, 71, 81].

Materials most susceptible to damage from x-ray, γ-ray, and electron beam radiation include electronic semiconductors and organic polymers. Semiconductors are subject to interface surface deterioration which can affect electrical properties [18, 19, 77, 114, 117]. Damage requires that a radiation energy threshold be exceeded. Radiation damage to organic polymers is principally

[5] Informal communication with Dr. Richard Olson, The Boeing Co., Seattle, Wash.

due to ionization [15]. Covalent bonds are broken and subsequent recombination of the free radicals is random and can lead to scission and cross-linking. Damage is generally directly proportional to dose.

The technical feasibility of spacecraft radiation sterilization in a program which includes careful selection of electronic parts, organic materials, manufacturing processes, and screening tests has been investigated in detail [10]. Further study is necessary to evaluate the economics of spacecraft radiation sterilization and to compare its effectiveness and applicability to currently used techniques.

Thermal sterilization. Terrestrial microorganisms are susceptible to high temperatures. Autoclaving, a standard sterilizing process in industry and food preparation, utilizes high temperature steam (dry heat) as the active ingredient.

Space scientists in the US currently consider dry-heat sterilization the most suitable means of decontaminating space hardware. Dry heat effectiveness depends upon:

- temperature
- time
- water activity
- open or closed system
- physical and chemical properties of the microorganism
- adjacent support material
- surrounding atmosphere

A simple logarithmic model [85], useful in defining conservative operational characteristics, expresses microbial destruction as a function of time and temperature:

$$\log N_U = \frac{-U}{D_T^z} + \log N_o \qquad (2)$$

where N_o is the initial microbial population, D_T^z is the time in units of time required to reduce the population by 90% at temperature T and for a temperature coefficient z, and N_U is the expected mean population after U units of time of heating.

Microbial heat inactivation, however, is more complex than this simplified model suggests and a rigorous treatment must consider water activity, population mix, and equilibration characteristics. The effect of a mixed population of several microbial groups, each with different initial population levels and D-values, is to produce a composite inactivation characteristic which tails out as destruction progresses. This increasing resistance to destruction necessitates close examination of the composite inactivation characteristic when high levels of decontamination are to be achieved.

Open and closed systems represent the two extremes in water movement to or from the microorganisms during heating. In closed systems, characterizing encapsulated and, in some cases, mated surface contamination, the microorganisms are located so that they are completely surrounded by a solid material impervious to water vapor transmission. In open systems, characterizing surface contamination, the microbes are in intimate and continuous contact with the surrounding atmosphere while they undergo dry-heat sterilization. Mated surface organisms, depending upon their physical situation and the degree of water transmission, are in either open or closed systems. The water vapor content immediately surrounding the organisms is all-important in this biocidal process, and the nature of the remainder of the surrounding gas atmosphere has little effect on the rate of dry-heat sterilization.

In the temperature range of 100°–125° C, inactivation is strongly dependent upon the relative humidity (RH), with lethality above 50% RH and below 0.2% RH much greater than the kill rates in the intermediate range [16]. This dramatic effect [7] of water activity upon inactivation of *Bacillus subtilis* at 125° C is shown in Figure 4.

For efficient sterilization, either the wet or dry extremes should be chosen. From a reliability standpoint, wet sterilization, although used earlier in the USSR [126], is felt unsuitable for delicate components; hence, the dry end of this spectrum has been selected for use by US flight projects. Studies are currently underway to determine a realistic minimum RH achievable during sterilization of typical spacecraft to take maximum advantage of this effect. Because of the heat sensitivity of much spacecraft material, the dry thermal sterilization temperature must be carefully selected. Recent

research [126] indicates that temperatures of 110–140° C are best suited for spacecraft sterilization. The US space program approves temperatures of 100° C and above and provides the set of specifications given in Table 4 for dry heat use [80].

Other factors involved in the design of a dry-heat sterilization process are the thermodynamic characteristics of the space vehicle and the heating environment, the numbers of organisms to be killed, and the distribution of the microbial burden on the spacecraft. Many factors in the design and configuration of the space vehicle strongly influence the temperature profile of the vehicle. Three of these factors are: location of the thermal control paths, placement of thermal insulation, and the physical properties of constituent materials.

In one of several alternative methods for applying heat to the vehicle, a canister containing the lander is sealed and the unit placed in an oven and heated. Heat flows from the atmosphere in the oven through the canister to the lander. In another more favored system, sterile gas is heated in a heat exchanger and recirculated through the canister. To optimize the thermal process, the heat lethality above the US specified minimum limit of 100° C should be integrated over the sterilization cycle—heat-up, temperature maintenance, and cool-down. In order to satisfy a wide range of designs, it is necessary to establish a range of processing conditions, all of which will produce the desired decontamination of a space lander. Clearly, the final selection of the sterilization process must be the result of a trade-off study between spacecraft design factors and process parameters.

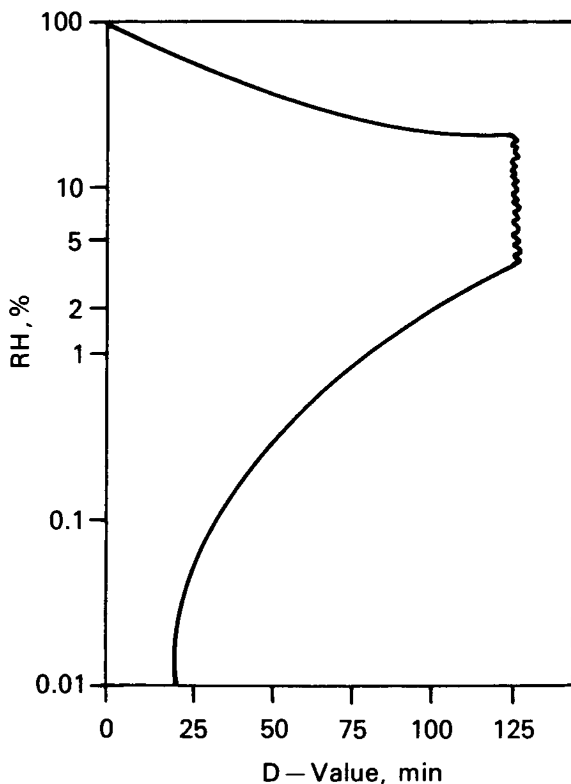

FIGURE 4.—Heat inactivation at 125° C as a function of R.H.

Nonsterile vehicles and components that have served their useful life, such as flyby or orbiting buses, can, theoretically, be sterilized by the activation of on-board exothermic heating agents such as Thermit, a mixture of aluminum powder and iron oxide. When ignited, it generates high sterilizing temperatures. However, using this technique forces basic spacecraft design constraints to ensure transfer of sufficient heat to all parts of the vehicle before the heat paths are destroyed, and imposes a weight penalty.

Sterilization at the end of a lander capsule's useful life would be too late to protect life-detection experiments or to prevent release of terrestrial life on a planet, eliminating the method from serious consideration.

Thermoradiation. Thermoradiation is attractive for contamination control [96] in spacecraft assembly because it exposes components to less heat than dry heat sterilization and less radiation than radiation alone.

The lethal effects of biologic systems of combined heat and ionizing radiation have been observed by many experimenters. Radiation sensitivity increases with a rise in temperature in such varied biologic systems as proteins [9, 20, 93, 94], viruses [1, 27, 55, 92, 124], spores [56, 57, 97, 113, 126], bacteria [62, 63] yeast [8], paramecia [94], insects [39], vertebrate cells [69, 118], and human tumors [2, 23, 27, 40, 50, 54, 55, 68, 98, 133, 134]. Sterilization in a composite heat and radiation environment can be ascribed to in-

creased radiation sensitivity. Temperatures at which the increased rates of inactivation occur are below those temperatures at which thermal inactivation becomes effective.

To evaluate the lethal synergism of heat and radiation, Sandia Laboratories[6] conducted experiments in the temperature range of 60°–125° C with radiation doses believed acceptable for typical spacecraft materials and components.

The sterilization effectiveness of ionizing radiation, dry heat, and then the simultaneous application of ionizing radiation and dry heat at various temperatures were compared. Figure 5, typical of these experiments, compares the inactivation of these sterilization agents singly, and in combination [104]. Additional experiments have revealed that this synergistic relationship of heat and radiation exists at temperatures as low as 60° C. Thermoradiation *D*-values at a dose rate of 8 krad/h varied from 1.5 h at 105° C to 3 h at 90° C and 6 h at 69° C [104]. These *D*-values represent a rather significant reduction in time required for sterilization. For example, the dry-heat *D*-values for *B. subtilis* var. *niger* at 60° C range from 53 to 274 h [130], depending on the moisture condition of the spores.

Graikoski [42], working with gamma radiation from Cobalt-60 and with several sporeforming species, concluded that a common mechanism is probably responsible for spore survival and radiosensitization. He postulated that the nucleoprotein deoxyribonucleic acid (DNA) component is important in this regard.

Samoylenko and Ivanov [108], comparing the sensitivity of bacteria to γ-irradiation and heat (50° C), showed that the radioresistant cultures tested were also more thermal-resistant. The DNA composition of the resistant strains had greater mole percent fractions of adenine and thymine than nonresistant strains.

The correlation of radiosensitivity with nucleic acid content has been verified by Sandia [28]; this correlation provides further evidence that the nucleic acid component of biosystems is responsible for the intrinsic radiosensitivity of a given biosystem.

[6] Thermoradiation—Experimentation Task in Contract W–12, 853. *Technical Assistance (Systems Analysis and Clean Room Monitoring) for Planetary Quarantine Program.*

Autosterilization. An autosterilizing material is one which contains an ingredient toxic to microorganisms. Studies by Astafyeva et al [8] have shown that various materials used in spacecraft, as well as oxide films produced under industrial conditions, possess autosterilizing properties. Such materials include alloys of magnesium, aluminum, copper, silver, surfaces covered with silver or copper, and certain types of rubber. There are also enamels which can cause reductions in microbial infection tens of thousands of times; however, test cultures remain viable beneath the coatings.

According to the data of Shank et al (1962) [112], Zsolnai (1962) [135], Godding and Lynch (1965) [41], the liquid fuels used in spacecraft

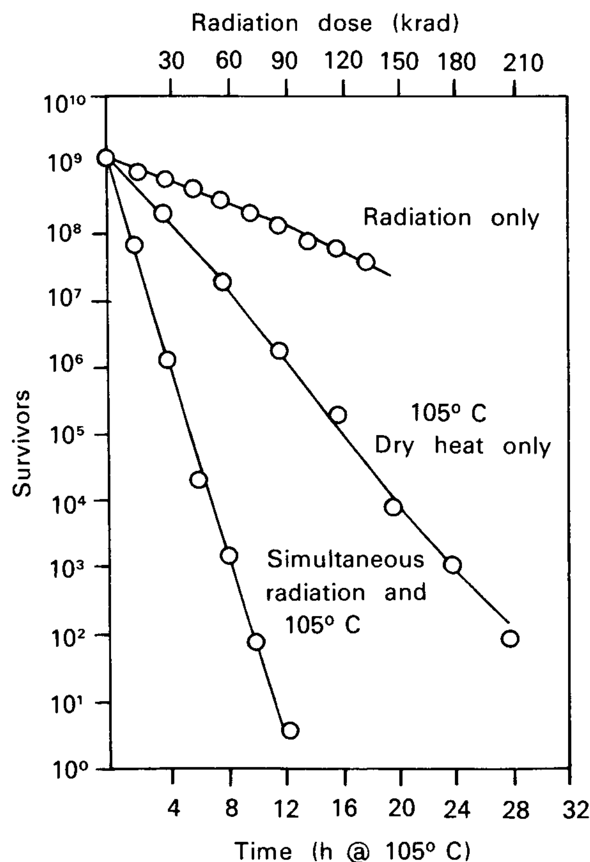

FIGURE 5.—Comparison of inactivation of *Bacillus subtilis* var. *niger* using γ-radiation at room temperature, dry heat alone at 105° C, then simultaneous dry heat and γ-radiation [104].

have autosterilizing properties on bacteria to varying degrees, while the solid fuel components do not.

The data of Opfell [83] indicate that when small amounts of paraformaldehyde are added to dyes, plastics, greases, and other compounds, they acquire bactericidal and autosterilizing properties. Willard and Alexander [131] showed that the addition of 3–7% formaldehyde to various dyes and coatings confers bactericidal properties on these materials. In order to obtain bactericidal cellulose cloth for a spacecraft, Vashkov and Shcheglova [128] chemically combined the macromolecule of cellulose with various bactericidal metals, quaternary-ammonium bases, and phenol preparations. These materials produced death in 70–100% of the gram-positive and gram-negative microorganisms placed on their surfaces.

Heat generated in manufacturing or testing parts can also produce internal sterility of parts. An example is the heat generated by exothermic reactions in polymerization.

In decontaminating spacecraft, difficulties have been encountered frequently because certain materials cannot tolerate the doses of radiation or temperatures that would ensure the necessary degree of sterility. Therefore, autosterilizing materials are of considerable interest in planetary quarantine and this characteristic can be evaluated during materials selection accompanying spacecraft design.

VERIFICATION PROCEDURES

The success of a spacecraft contamination control program is measured by enumeration of the microbial levels, especially bacterial spores, in and on space hardware. Although this general approach is practiced in other fields, spacecraft sterilization presents unique problems. In the food and pharmaceutical industries, for example, sterility tests can be performed on statistically significant samples of the final product. On spacecraft, where large numbers of items do not exist, a postcycle sterility test would be inconclusive, could produce recontamination, and might degrade flight hardware. Consequently, presterilization assays are used in combination with laboratory-verified inactivation characteristics in estimating the limit of post-sterilization contamination levels. This process requires knowledge of the microbial population mix on the spacecraft to assure that the inactivation characteristics for the sterilization technique selected do not radically differ from the laboratory values. Microbial samples, collected by flight project personnel, are cultured and counted by an assay laboratory certified for this purpose.

A manual describing assay procedures, published by NASA in 1967, revised 1968, specifies techniques for assay of space hardware and intramural environments used for assembly and testing. The general procedure for enumerating aerobic and anaerobic microorganisms and spores is illustrated in Figure 6.

Most assay systems for spore enumeration involve heating the microbial suspension prior to plating, which is referred to as heat shock or heat activation. It has been shown repeatedly that many spores require such treatment for maximal germination. Counts of one variety of *Bacillus subtilis* have shown two- to threefold increases with heat shock [31]. However, certain spores germinate without heat shock and some can be adversely affected by heat shock. For example, viable counts of *Bacillus subtilis* var. *niger* spores are consistently reduced two to three times after heat shocking. In a mixed, naturally occurring spore population (the type of contamination with which spacecraft assays are concerned), some spores may not survive heat shock, others would survive, and still others would be stimulated.

Research in this area is in two main directions. The Center for Disease Control Laboratory in Phoenix has suggested that an ideal system for assaying spores in a mixed microbial population would be a technique that utilizes a nonthermal treatment for killing vegetative cells without harming spores, and a nontoxic chemical stimulus to induce maximal spore germination. The procedure for enumerating anaerobic microorganisms is essentially the same as that for aerobic microorganisms, with the exception that the culture plates are incubated under strict anaerobic conditions. Experience has shown that there are very few strictly anaerobic bacteria on spacecraft. Hence, this procedure is seldom used.

Organism enumeration, knowledge of the effects of sterilants used, and physical measurement of the parameters affecting the sterilants applied to the spacecraft are the bases on which the PQ authority can confidently certify that the mission, when launched, will comply with COSPAR requirements. Verification of flight project compliance with the PQ requirements enables each launching nation to assure concerned organizations that biologic safeguards have been established, and that a course is being followed which will result in the planets being maintained as biologic preserves for scientific investigations.

OUTLOOK FOR THE FUTURE

Throughout the recorded history of planet Earth, invasions of a species of plant or animal often resulted in regional changes of catastrophic proportions. Early man often interposed crude barriers, frequently only distance, between the invader and himself when he was the susceptible host. Developing trade and commerce in the world bridged natural barriers to life forms. The time has come to consider insuring the maintenance of interplanetary barriers.

The huge and presumably hostile space between the planets has served as a natural

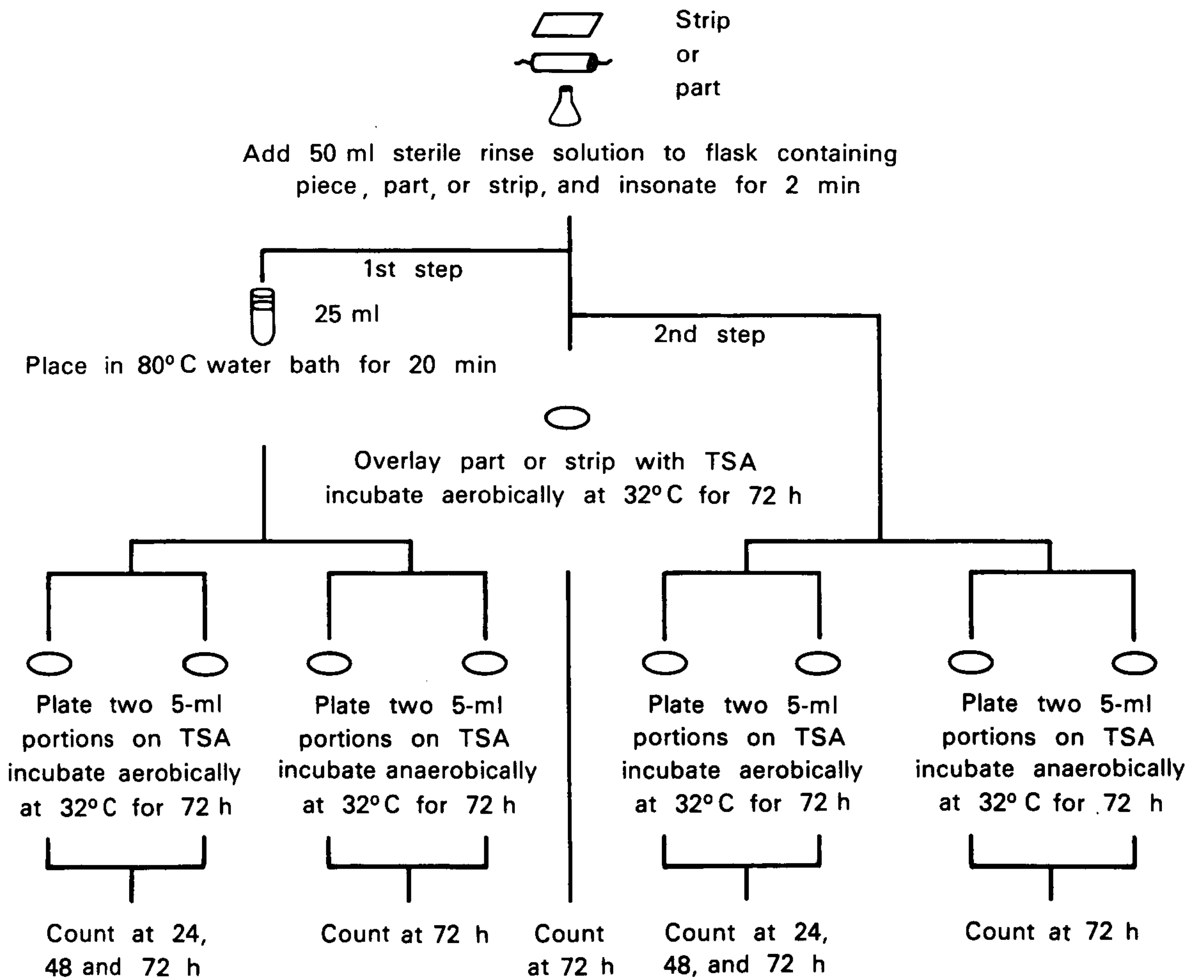

FIGURE 6.—Schematic of piece, part, and fallout strip analysis.

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barrier throughout the ages to prevent the transfer of pathogenic agents, if they exist, between the celestial bodies. With the advent of space travel this natural barrier has been transcended. An artificial barrier, planetary quarantine, has now been instituted to effect the required degree of protection.

The US planetary exploration program has been guided by NASA's Planetary Quarantine Program. In accordance with international agreements, the COSPAR has been kept informed of the measures employed to control contamination within the specified limits for each spacecraft launch.

As space exploration programs have advanced, knowledge has been accumulated regarding the biological and physical characteristics of the planets. We are now quite confident of the estimates of the environmental characteristics of Venus, and similar data concerning Mars are being assimilated from flights of Mars 1, Mars 2, and Mariner 9.

Such knowledge has resulted in reducing the uncertainty in the estimation of key PQ parameters and in supporting the relaxation of PQ constraints. For example, following the flight of Venera 7, a 5-decade reduction in the value of the probability of growth on the surface of the planet was effected in the light of the very high temperatures found to exist on the surface. Quarantine has now been lifted in the US program from Mercury and from Venus, with the exception of possible microenvironments in the atmosphere of the latter.

It is expected that similar modifications will follow as space exploration proceeds. The forthcoming martian landings by the space launching nations should provide insight into the important question of the availability of water in the microenvironments on the martian surface. With these data, the requirement for quarantine of Mars can be realistically reviewed. The Pioneer missions to Jupiter may provide insight into the existence and abundance of similar life-supporting factors for the Jovian planets and their satellites, hence bear upon the issue of quarantine for these bodies.

The success of planetary quarantine can be assured only by rigorous measures, to make sure that no errors have been made and that no factor has been overlooked. Only by the use of such rigid measures, burdensome as they may be, can the planets be undisturbed awaiting future scientific investigations.

The program of planetary quarantine conceived by the ICSU, and administered by proper authorities within the launching nations, includes rigorous measures and combines the scientific and technical knowledge necessary to formulate requirements with the authority needed to ensure compliance. Thus, PQ is safeguarding the planets of our solar system from possible irreversible loss associated with our newly developed exploration ability. Until such time as space exploration can show that quarantine is no longer necessary, planetary spacecraft must continue to be governed by quarantine requirements. In this way, mankind can continue the exploration of space, confident that the threat of irreversible contamination of the planets has been contained and that their environments will remain pristine awaiting the coming of mankind and the uses, in the dim future, that man may design for these planets.

REFERENCES

1. ADAMS, W. R., and E. C. POLLARD. Combined thermal and primary ionization effects on a bacterial virus. *Arch. Biochem. Biophys.* 36:311–322, 1952.
2. ALTMAN, K. I., G. B. GERBER, and S. OKADA. Radiation effects on molecules. *In*, Okada, S. *Radiation Biochemistry*, Vol. 1, pp. 1–76. New York, Academic, 1970.
3. American Institute of Biological Sciences (AIBS). *Minutes, Semi-Annual NASA Spacecraft Sterilization Technology Seminar.* Washington, D.C., AIBS, 1972
4. ANGELOTTI, R., K. H. LEWIS, et al. *Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components.* Cincinnati, USPHS, Robt. A. Taft Sanit. Eng. Cent., 1965. (First quart. rep.)
5. ANGELOTTI, R., K. H. LEWIS, et al. *Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components.* Cincinnati, USPHS, Robt. A. Taft Sanit. Eng. Cent., 1965. (Second quart. rep.)
6. ANGELOTTI, R., K. H. LEWIS, et al. *Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components.* Cincinnati, USPHS, Robt. A. Taft Sanit. Eng. Cent., 1966. (Fifth quart rep.)
7. ANGELOTTI, R., J. H. MARYANSKI, T. F. BUTLER, J. T. PEELER, and J. E. CAMPBELL. Influence of spore

UCIBILITY OF THE
PAGE IS POOR

moisture content on the dry heat resistance of *Bacillus subtilis* var. *niger*. *Appl. Microbiol.* 16(5): 735–745, 1968.

8. ASTAFYEVA, A. K., V. I. VASHKOV, E. N. NIKIFOROVA, and N. V. RAMKOVA. Methods for a spacecraft sterilization. *In*, Brown, A. H., and F. G. Favorite, Eds. *Life Sciences and Space Research*, Vol. 5, pp. 38–43. Amsterdam, North-Holland, 1967.
9. AUGENSTEIN, L. G., T. BRUSTAD, and R. MASON. The relative roles of ionization and excitation processes in the radiation inactivation of enzymes. *In*, Augenstein, L. G., R. Mason, and H. Quastler, Eds. *Advances in Radiation Biology*, Vol. 1, pp. 227–266. New York, Academic, 1964.
10. BARRETT, M. J., and W. C. COOLEY. *On the Feasibility of Radiation Sterilization of Planetary Spacecraft*. Washington, D.C., Exotech Inc., 1966. (TR–012)
11. BARTHOLOMEW, C. S., and D. C. PORTER. Reliability and sterilization. *J. Spacecr. Rockets.* 3(12):1762–1766, 1966.
12. Battelle Memorial Institute. *Radiation Effects State-of-the-Art*, 1964–1965 (Hamman, D. J., E. N. Wyler, R. K. Thatcher, W. H. Veagie, Jr., F. R. Shober, et al). Columbus, Ohio, Battelle Mem. Inst., 1965. (REIC–38)
13. BLICKMAN, B. I., and T. B. LANAHAN. Ventilated work cabinets reduce lab risks. *Saf. Maint.* 120:34–36, 44–45, 1960.
14. BOEING CO. *The Effects of Ethylene Oxide upon Operating Electronic Devices with Breached Hermetic Seals.* Seattle, Boeing Co., 1967. (Rep. D2–36527–1)
15. BOLT, R. O., and J. G. CARROLL, Eds. *Radiation Effects on Organic Materials.* New York, Academic, 1963.
16. BRANNEN, J. P., and D. M. GARST. Dry heat inactivation of *Bacillus subtilis* var. *niger* spores as a function of relative humidity. *Appl. Microbiol.* 23(6):1125–1130, 1972.
17. BROWN, A. H. *Conference on Potential Hazards of Back Contamination from the Planets.* Washington, D.C., Nat. Acad. Sci. USA, Space Sci. Board, 1965.
18. BROWN, R. R. Proton and electron permanent damage in silicon semi-conductor devices. *In, Radiation Effects in Electronics.* Philadelphia, Am. Soc. Test. Mat., 1964. (Spec. Tech. Publ. No. 384)
19. BRUCKER, G., W. DENNEHY, and A. HOLMES-SIEDLE. High energy radiation damage in silicon transistors. *IEEE Trans. Nucl. Sci.* NS–12(5):69–71, 1965.
20. BRUSTAD, T. Heat as a modifying factor in enzyme inactivation by ionizing radiations. *In, Biological Effects of Neutron and Proton Irradiations*, Vol. 2, pp. 404–410. Vienna, Int. At. Energy Agency, 1964.
21. CLEMEDSON, C.-J. Sterilization of lunar and planetary space vehicles. *XIII Int. Astronaut. Congr.* (Varna, 1962), Vol. 1, pp. 292–313. Vienna-New York, Springer, 1964.
22. COSPAR Study Group on Standards for Space Probe Sterilization. The value of agreed standards of sterility (Heden, C. G.). Appendix B. Tentative nomenclature and analytical basis for use in planetary quarantine. *COSPAR Tech. Man. Ser.*, No. 4, pp. 29–34. Paris, Muray Print, 1968.
23. CONCER, A. D., and M. L. RANDOLPH. Magnetic centers (free radicals) produced in cereal embryos by ionizing radiation. *Radiat. Res.* 11:54–66, 1959.
24. CETEX. Contamination by extraterrestrial exploration. *Nature* 183:925–928, 1959.
25. CETEX. Development of international efforts to avoid contamination of extraterrestrial bodies. *Science* 128:887–889, 1958.
26. CRAVEN, C. W. Planetary quarantine analysis, Part 1. *Astronaut. Aeronaut.* 6(8):20–24, 1968.
27. DIGIOIA, G. A., J. J. LICCIARDELLO, J. T. R. NICKERSON, and S. A. GOLDBLITH. Effect of temperature on radiosensitivity of Newcastle disease virus. *Appl. Microbiol.* 19:455–457, 1970.
28. DUGAN, V. L., and R. TRUJILLO. Synergistic inactivation of viruses by heat and ionizing radiation. *Biophys. J.* 12:92–113, 1972.
29. EVANS, C. W., W. M. SMITH, E. A. JOHNSON, and E. R. GIBLETT. Bacterial flora of the normal human skin. *J. Invest. Dermatol.* 15:305–324, 1950.
30. Exotech Systems, Inc. *Planetary Quarantine Specification Sheets.* Issued by direction of NASA Planet. Quar. Off. Washington, D.C., Exotech Syst., 1972.
31. FAVERO, M. S. *In*, Hall, L. B., Ed. *Planetary Quarantine: Principles, Methods, and Problems*, pp. 27–36. New York, Gordon and Breach, 1971.
32. FAVERO, M. S., J. J. McDADE, J. A. ROBERTSEN, R. K. HOFFMAN, and R. W. EDWARDS, Microbiological sampling of surfaces. *J. Appl. Bacteriol. 31:336*–343, 1968.
33. FAVERO, M. S., and N. J. PETERSON. *Recovery of Surface and Buried Contamination.* Presented at AIBS/NASA Semi-Ann. NASA Spacecr. Steriliz. Technol. Semin., San Francisco, July 1972.
34. FEDEROVA, R. I. Possibility of the spreading of viable germs in outer space. *In*, Imshenetskiy, A. A., Ed. *Extraterrestrial Life and Its Detection Methods*, pp. 154–167, Washington, D.C., NASA, 1971. (NASA TT–F–710)
35. FRANK, M., C. D. TAULBEE, and H. L. CHAMBERS. Influence of operating conditions on radiation damage to transistor gain. *In, Radiation Effects in Electronics.* Philadelphia, Am. Soc. Test. Mat., 1964. (Spec. Tech. Publ. No. 384)
36. GEIGER, P. J., F. A. MORELLI, and H. P. CONROW. Effects of ultrahigh vacuum on three types of microorganisms. *In, Space Programs Summary*, Vol. IV, pp. 109–115. Pasadena, Calif., Jet Propul. Lab., 1964. (JPL 37–27)
37. General Electric Company. *The Effects of Ethylene Oxide Sterilization in Typical Spacecraft Materials.* Program Information Request Release. N.D.
38. General Electric Co. *A Research Study To Definitize a Bio-Isolator Suit System* (BISS). Philadelphia, General Electric Co., 1967. (Final Rep. 67SD888)
39. GINOZA, W. The effect of ionizing radiation on nucleic acids of bacteriophages and bacterial cells. *Ann. Rev. Microbiol.* 21:325–368, 1967.

40. GINOZA, W. Inactivation of viruses by ionizing radiation and by heat. *In*, Maramorosch, K., and H. Koprowski, Eds. *Methods in Virology*, Vol. IV, pp. 139–209. New York, Academic, 1968.
41. GODDING, R. M., and V. H. LYNCH. Viability of *Bacillus subtilis* spores in rocket propellants. *Appl. Microbiol.* 13(1):10–14, 1965.
42. GRAIKOSKI, J. T. The simultaneous lethal effect of temperature and gamma radiation on bacterial spores. *Diss. Abstr.* 22(2):394, 1961.
43. GREENE, V. W., B. WALKER, Jr., and O. A. ANDERSON. *Methodology of Measuring Internal Contamination in Spacecraft Hardware.* Minneapolis, Univ. Minn., Sch. Public Health, 1967. (Final Rep.)
44. GREMILLION, G. G. The use of bacteria-tight cabinets in the infectious disease laboratory. *In, Proceedings, Second Symposium Gnotobiotic Technology*, pp. 171–182. Terre Haute, Ind., Notre Dame Univ. Press, 1960.
45. HAGAN, C. A., J. F. GODFREY, and R. H. GREEN. The effect of temperature on the survival of microorganisms in deep space vacuum. *Space Life Sci.* 3(2): 108–117, 1971.
46. HALL, L. B. *A Decade of Development in Sterilization Technology by the United States Space Program.* Presented at Int. Steriliz. Conf., Amsterdam, Sept. 1972.
47. HALL, L. B. The importance of sterilization techniques in space exploration. *COSPAR Tech. Man.*, No. 4, pp. 3–18. Paris, Muray Print, 1968.
48. HALL, L. B., and M. J. HARTNETT. Measurement of the bacterial contamination on surfaces in hospitals. *Public Health Rep.* 79(11):1021–1024, 1964.
49. HILL, L. W. *Quantitation of Buried Contamination by Use of Solvents.* Fargo, N.D., Univ. N. Dak., 1972.
50. HOFF, A. J., and D. C. KONINGSBERGER. Production of free radicals in DNA and inactivation of its biological activity by gamma-rays. *Int. J. Radiat. Biol.* 17(5): 459–465, 1970.
51. HOFFMAN, A. R. Microbial burden prediction model. *In, Planetary Quarantine* (Semi-ann. rev., space res. and technol., July–Dec., 1970). Pasadena, Calif., Jet Propul. Lab., 1971. (Doc. 900–484)
52. HOFFMAN, A. R., and R. J. REICHERT, *Mariner Mars 1971 Planetary Quarantine Plan*, Part 1. Pasadena, Calif., Jet Propul. Lab., 1970. (JPL Rep. PD 610–18)
53. HOFFMAN, A. R., and D. A. WINTERBURN. *Microbial Burden Prediction Model for Unmanned Planetary Spacecraft.* Pasadena, Calif., Jet Propul. Lab., 1972. (JPL Rep. 900–566)
54. HORAN, P. K., and W. SNIPES. The temperature dependence of radiation-induced free-radical destruction. *Int. J. Radiat. Biol.* 19(1):37–43, 1971.
55. HOTZ, G., and A. MÜLLER. The action of heat and ionizing radiation on the infectivity of isolated ϕx–174 DNA. *Proc. Natl. Acad. Sci. USA* 60:251–257, 1968.
56. HUNT, R. E., and R. R. ERNST. *A Study of the Requirements, Preliminary Concepts and Feasibility of a New System to Process Medical/Surgical Supplies in the Field*, Vol. 1 (Technical Summary), Part 1 (Technical Investigation). Cambridge, Mass., Arthur D. Little, 1971. (Final rep., Phase 1 No. 72688; Contr. DADA–17–70–C–0072)
57. HUNT, R. E., and R. R. ERNST. Analysis of candidate sterilizers and sterilization processes. *In, A Study of the Requirements, Preliminary Concepts, and Feasibility of a New System to Process Medical/Surgical Supplies in the Field*, Vol. 1 (Technical Summary). Appendix 6.2. Cambridge, Mass., Arthur D. Little, 1971. (Final rep. 72688, Phase 1; Contr. DADA 17–70–C–0072)
58. IMSHENETSKIY, A. A., and S. ABYZOV. Sterilization of spacecraft. *In*, Imshenetskiy, A. A., Ed. *Extraterrestrial Life and Its Detection Methods*, pp. 230–252. Moscow, Nauka, 1970. (NASA TT–F–710)
59. IMSHENETSKIY, A. A., and S. V. LYSENKO. Effect of a high vacuum on microorganisms. *In*, Imshenetskiy, A. A., Ed. *Extraterrestrial Life and Its Detection Methods*, pp. 129–143. Washington, D.C., NASA, 1972. (NASA TT–F–710)
60. IMSHENETSKIY, A. A., S. S. ABYZOV, G. T. VORONOV, L. A. KUZJURINA, and S. V. LYSENKO. *In*, Brown, A. H., and F. G. Favorite, Eds. *Life Sciences and Space Research*, Vol. 5, pp. 250–260. Amsterdam, North-Holland, 1967.
61. INGRAM, G. E., M. A. MARTIN, E. BERGER, and T. F. GREEN, *Voyager Mars Planetary Quarantine—Basic Math Model Report.* Philadelphia, General Electric Co., 1967. (Rep. VOY–C2–TR8)
62. JAFFE, L. D. Sterilizing unmanned spacecraft. *Aeronaut. Aerosp. Eng.* 1:22–29, 1963.
63. JAFFE, L. D. Problems in sterilization of unmanned space vehicles. *In*, Florkin, M., and A. Dollfus, Eds. *Life Sciences and Space Research II*, pp. 406–432. Amsterdam, North-Holland, 1964.
64. Jet Propulsion Laboratory. *Effects of Decontamination and Sterilization on Spacecraft Polymetric Materials* (Lee, S. M., and J. J. Licari), Pasadena, Calif., Jet Propul. Lab., 1968. (NASA CR–94312)
65. KAPELL, G. F., J. J. McDADE, and T. R. GAVIN. *Experimental Assembly and Sterilization Laboratory (EASL) Operations: Phase I.* Pasadena, Calif., Jet Propul. Lab., 1966. (Tech. Rep. TR32–941)
66. KAUTZ, G. P., and P. TARVER. Plan for sterilization of Voyager capsule. *In, Spacecraft Sterilization Technology*, pp. 559–567. Washington, D.C., NASA, 1966. (NASA SP–108)
67. KOHORST, D. P., and H. HARVEY. Polymers for use in sterilized spacecraft. *In, Spacecraft Sterilization Technology*, pp. 327–342. Washington, D.C., NASA, 1966. (NASA SP–108)
68. KÜRZINGER, K. Temperature-dependence of radiation sensitivity in the dry state: a model derived from experiments using atomic hydrogen II. *Int. J. Radiat. Biol.* 19(1):45–50, 1971.
69. LEA, D. E. *Actions of Radiations on Living Cells*, 416 pp. London, Cambridge Univ. Pr., 1955.
70. LEDOUX, F. N. *Decontamination of the AIMP–D Space-*

craft. Greenbelt, Md., NASA/Goddard Space Flight Cent., 1967. (Doc. X-723-67-171)

71. LEVY, P. W. Radiation effects in glass and other materials. *Phys. Today* 15(9):19–23, 1962.
72. LOVELL, D. L. Skin bacteria; their location with reference to skin sterilization. *Surg. Gynecol. Obstet.* 80(2):170–174, 195, 1945.
73. LYLE, R. G., and I. JACOBY. *Estimation of Encapsulated Microbial Burden.* Washington, D.C., Exotech Syst., 1972. (TR 72-13)
74. McDADE, J. J. Control of microbial contamination. *In,* Hall, L. B., Ed. *Planetary Quarantine: Principles, Methods, and Problems,* pp. 37–62. New York, Gordon and Breach, 1971.
75. McDADE, J. J., M. S. FAVERO, G. S. MICHAELSEN, and D. VESLEY. Environmental microbiology and the control of microbial contamination. *In, Spacecraft Sterilization Technology,* pp. 51–86. Washington, D.C., NASA, 1966. (NASA SP–108)
76. McNALL, E. G., W. T. DUFFY, and J. J. IANDOLO. Microbiological techniques for recovery from interiors of solids. *In, Spacecraft Sterilization Technology,* pp. 155-176. Washington, D.C., NASA, 1966. (NASA SP–108)
77. MESSENGER, G. C. Displacement damage in silicon and germanium transistors. *IEEE Trans. Nucl. Sci.* NS–12(2):53–65, 1965.
78. MEYER, A., and E. SEITZ. *Ultraviolet Radiation. Production, Measurement and Use in Medicine, Biology and Technology.* Moscow, Inostrannay Lit., 1952.
79. NASA. *NASA Standards for Clean Rooms and Work Stations for the Microbially Controlled Environment.* Washington, D.C., NASA, 1967. (NHB 5340.2)
80. NASA. *Planetary Quarantine Provisions for Unmanned Planetary Missions.* Washington, D.C., NASA, 1969. (NHB 8020.12)
81. National Bureau of Standards. *Shielding for High-Energy Electron Accelerator Installations.* Washington, D.C., Natl. Bur. Stand., 1964. (NBS Handb. 97) (Presently available from Natl. Counc. Rad. Prot. & Meas., Bethesda, Md., No. 31)
82. OLSON, R. L., and S. J. FRASER. *Release of Microorganisms from Solids after Simulated Hard Landings.* Seattle, Boeing Co., 1970. (Final rep.)
83. OPFELL, J. B. A general review of chemical sterilization in space research. *In,* Florkin, M., and A. Dollfus, Eds. *Life Sciences and Space Research II,* pp. 385–405. Amsterdam, North-Holland, 1964.
84. PAIK, W. W., and J. A. STERN. *The Microbiological Aspects of Sterilization Assembly Development Laboratories EASL and SADL.* Pasadena, Calif., Jet Propul. Lab., 1968. (Tech. Rep. TR 32–1207)
85. PFLUG, I. J. Sterilization of space hardware. *Environ. Biol. Med.* 1:63–81, 1971.
86. PHILLIPS, C. R. Gaseous sterilization. *In,* Lawrence, C. A., and S. S. Block, Eds. *Disinfection, Sterilization and Preservation.* Philadelphia, Lea & Febiger, 1968.
87. PHILLIPS, C. R., and R. K. HOFFMAN. Sterilization of interplanetary vehicles. *Science* 132:991–995, 1960.
88. PHILLIPS, G. B. Microbiological barrier techniques. Ft. Detrick, Md., US Army Biol. Labs., 1965. (Tech. Manuser. 260)
89. PHILLIPS, G. B. Microbiological barrier techniques. *In, Spacecraft Sterilization Technology,* pp, 105–135. Washington, D.C., NASA, 1966. (NASA SP–108)
90. PHILLIPS, G. B., and E. HANEL, Jr. Use of ultraviolet radiation in microbiological laboratories. *US Gov't. Res. Rep.* 34:122, 1960.
91. PHILLIPS, G. B., F. E. NOVAK, and R. L. ALG. Portable inexpensive plastic safety hood for bacteriologists. *Appl. Microbiol.* 3:216–217, 1955.
92. POLLARD, E. C. The action of ionizing radiation on viruses. *In,* Smith, K. M., and M. A. Lauffet, Eds. *Advances in Virus Research,* Vol. 2, pp. 109–151. New York, Academic, 1954.
93. POLLARD, E. C. Thermal effects on protein, nucleic acid and viruses. *In,* Duchesne, J., Ed. *Advances in Chemical Physics. Vol. 7: The Structure and Properties of Biomolecules and Biological Systems,* pp. 201–237, New York, Interscience, 1964.
94. POLLARD, E. C., and W. SOLOSKO. The thermal inactivation of T_4 and λ bacteriophage. *Biophys. J.* 11(1): 66–74, 1971.
95. PORTNER, D. M., D. R. SPINER, R. K. HOFFMAN, and C. R. PHILLIPS. Effect of ultrahigh vacuum on viability of microorganisms. *Science* 134:2047, 1961.
96. POTTER, R. C., C. SCHNEIDER, M. RYSKA, and D. O. HUMMEL. Trends in radiation polymerization. *Angew. Chem. Engl.* 7:845–856, 1968.
97. [PRATT]. The cleansing light (by "M.S."). *In, MIT Reports on Research,* pp. 3–4. Cambridge, Mass., MIT, 1972.
98. PRYOR, W. A. Free radicals in biological systems. *Sci. Am.* 223(2):70–83, 1970.
99. PULEO, J. R., M. S. FAVERO, and N. J. PETERSON. Use of ultrasonic energy in assessing microbial contamination of surfaces. *Appl. Microbiol.* 15(11):1345–1351, 1967.
100. PULEO, J. R., M. S. FAVERO, and G. J. TRITZ. Feasibility of using ultrasonics for removing viable microorganisms from surfaces. *Contam. Control* 6(4):58–67, 1967.
101. PULEO, J. R., and G. S. OXBORROW. *Recovery of Viable Microorganisms from Solids: I. Model Systems.* Phoenix, Ariz., USPHS, 1966. (Natl. Commun. Dis. Cent. Rep. 13)
102. REDMANN, G. H. *Experimental Assembly and Sterilization/Sterile Assembly and Development Laboratory Test and Operation.* Pasadena, Calif., Jet Propul. Lab., 1967. (TM–33–322)
103. REED, L. I. Microbiological analysis techniques for spacecraft sterilization. *In, Space Programs Summary 37–32,* Vol. IV, pp. 35–42. Pasadena, Calif., Jet Propul. Lab., 1965.
104. REYNOLDS, M. C., and D. M. GARST. Optimizing thermal

and radiation effects for bacterial inactivation. *Space Life Sci.* 2:394–399, 1970.

105. Reynolds, O. E. Developments in the analysis of planetary quarantine requirements. *In*, Sneath, P. H. A., Ed. *Life Sciences and Space Research XI*, pp. 3–7. Berlin, Akademie, 1973.
106. Roark, A. L. *A Stochastic Approach to Bioburden Estimation and Prediction—A Preliminary Report.* Albuquerque, N. Mex., Sandia Labs., 1970. (SC–RR–70–561)
107. Sagan, C., and S. Coleman. Spacecraft sterilization standards and contamination of Mars. *J. Astronaut. Aeronaut.* 3(5):22–27, 1965.
108. Samoylenko, I. I., and K. K. Ivanov. Bacterial resistance to radiation and heat effects. *Zh. Mikrobiol. Epidemiol. Immunobiol.* (2):113–115, 1972.
109. Samoylova, K. A. *Deystviye Ul'trafioletovoy Radiatsii na Kletku* (Transl: *Effect of Ultraviolet Radiation on the Cell*). Leningrad, Nauka, 1967.
110. Schalkowsky, S., and I. Jacoby. *Safety Margins in the Implementation of Planetary Quarantine Requirements.* Washington, D.C. Exotech Syst., 1972. (Interim Rep. TR72–14)
111. Schalkowsky, S., and R. C. Kline, Jr. Analytical basis for planetary quarantine. *In*, Hall, L. B., Ed. *Planetary Quarantine: Principles, Methods and Problems*, pp. 9–26. New York, Gordon and Breach, 1971.
112. Shank, J. L., J. H. Silliker, and R. H. Harper. The effect of nitric oxide on bacteria. *Appl. Microbiol.* 10:185–189, 1962.
113. Shumaeva, Yu. F. *Use of Certain Surfactants for Purposes of Disinfection.* Moscow, 1966. (Candidate diss.)
114. Sonder, E., and L. C. Templeton. Gamma irradiation of silicon. *J. Appl. Phys.* 31(7):1279–1286, 1960; 36(6):1811–1815, 1965.
115. Stroud, R. H., and R. G. Lyle. *Contamination Control by Use of Ethylene Oxide.* Washington, D.C., Exotech Syst. (Tech. Summ. TR72–11)
116. Swenson, B. L. *Spacecraft Component Survivability during Entry into the Jovian Atmosphere.* Washington, D.C., NASA, 1971. (NASA TM–X–2276)
117. TRW Space Technology Laboratory. *The Energy Dependence of Electron Damage in Silicon.* Redondo Beach, Calif., TRW Space Technol. Lab., 1964.
118. Timofeyev-Risovskiy, N. W., and K. G. Zimmer. *Das Trefferprinzip in der Biologie* (Transl: *The Thrust Principle in Biology*), Bd. 1, Leipzig, Hirzel, 1947.
119. Trauth, C. A., Jr. A multistage decision model for mission non-contamination requirements. *Space Life Sci.* 1:135–149, 1968.
120. Trauth, C. A., Jr., and A. L. Roark. *Dry Heat Sterilization of Heterogeneous Bacterial Populations.* Presented to PQAP, March 1972.
121. Trexler, P. C. Flexible-wall plastic film isolators. *In, Proceedings, Second Symposium Gnotobiotic Technology*, pp. 55–60. Terre Haute, Ind., Notre Dame Univ. Pr., 1960.
122. Trexler, P. C. Germ-free isolators. *Sci. Am.* 211(1):78–84, 86, 88, 1964.
123. Trexler, P. C. The gnotobiote-review and future. *Bio-Med. Purv.* 1:47–58, 1961.
124. Trujillo, R. E. *Preparation and Assay of T4 Bacteriophage.* Albuquerque, N. Mex., Sandia Labs., 1971. (Tech. Rep. SC–RR–710107)
125. Ulrich, J. A. Skin carriage of bacteria in the human. *In, Spacecraft Sterilization Technology*, pp. 87–95. Washington, D.C., NASA, 1966. (NASA SP-108)
126. Vashkov, V. I. Modern methods and means of sterilization of spacecraft. *In*, Imshenetskiy, A. A., Ed. *Extraterrestrial Life and Its Detection Methods*, pp. 207-219. Moscow, Nauka, 1970. (NASA TT–F–710)
127. Vashkov, V. I., and A. G. Prishchep. Efficiency of sterilization by making use of ethylene oxide and methyl bromide mixture. *In*, Brown, A. H., and F. G. Favorite, Eds. *Life Sciences and Space Research*, Vol. V, pp. 44–50. Amsterdam, North-Holland, 1967.
128. Vashkov, V. I., and G. Shcheglova. Imparting antimicrobial properties to various materials. *In, COSPAR Tech. Manual*, No. 4, pp. 127–131. Paris, Muray Print, 1968.
129. Vashkov, V. I., N. V. Rashkova, and G. V. Shcheglova. Planetary quarantine. *In, Karantin Planet: Printsipy, Metody i Problemy, Osnovy Kosmicheskoy Biologii i Meditsiny* (Transl: *Principles, Methods, Problems, and Means of Sterilization of Spacecraft*), Vol. 1, Part 3, Chap. 4, pp. 3–156. Washington, D.C., NASA, 1970. (NASA TT-F-13769)
130. Vesley, D., G. Smith, J. Haugen, and Y. Thun. Survival of microbial spores under several temperature and humidity conditions. *In*, Pflug, I. J., Ed. *Environmental Microbiology as Related to Planetary Quarantine.* Minneapolis, Univ. Minn., Sch. Public Health, 1969. (Semi-Ann. Rep. 3)
131. Willard, M., and A. Alexander. A self-sterilizing coating for spacecraft surfaces. *Nature* 202:658–659, 1964.
132. Williams, R. E. O. Healthy carriage of *Staphylococcus aureus:* its prevalence and importance. *Bacteriol. Rev.* 27:56–71, 1963.
133. Zimmer, K. G. From target theory to molecular radiobiology. *Phys. Med. Biol.* 14(4):545–553, 1969.
134. Zimmer, K. G., L. Ehrenberg, and A. Ehrenberg. Nachweis langlebiger maanetischer zentren in bestrahlten biologischen medien und oaren bedeutung fur die strahlenbiologie. (Transl: Determination of long-lived magnetic centers in irradiated biological media and their significance for radiation biology.) *Strahlentherapie* 103:3–15, 1951.
135. Zsolnai, T. Versuche zur entdeckung neuer fungistatika–VI (Transl: Experiments for the development of new fungistatics). *Biochem. Pharmacol.* 11:995–1016, 1962.
136. Zwerling, S. *A research Study to Definitize a Bio-Isolator Suit System (BISS).* Philadelphia, General Electric Co., 1967. (Final Rep. 67SD660)

BIBLIOGRAPHY

ADAMS, M. H. *Bacteriophages*, 592 pp. New York, Interscience, 1959.

BRANNEN, J. P. A rational model for thermal sterilization of microorganisms. *In, Mathematical Biosciences*, Vol. 2 (1/2), pp. 165-179. New York. Elsevier, 1968.

DAVIS, N. S., G. J. SILVERMAN, and W. H. KELLER. Combined effects of ultra-high vacuum and temperature on the viability of some spores and soil organisms. *Appl. Microbiol.* 11(3):202-209, 1963.

DECKER, H. M., and L. M. BUCHANAN. Filter applications for spacecraft sterilization program. *In, Spacecraft Sterilization Technology*, pp. 259-268. Washington, D.C., NASA, 1966. (NASA SP-108)

DUGAN, V. L. A kinetic analysis of spore inactivation in a composite heat and gamma radiation environment. *Space Life Sci.* 2:498-505, 1971.

DUGAN, V. L. *Principles of Operation of the Vacuum Probe Microbiological Sampler.* Albuquerque, Sandia Labs., 1967. (SC-RR-67-688)

DUGAN, V. L., W. J. WHITFIELD, J. J. MCDADE, J. W. BEAKLEY, and F. W. OSWALT. *A New Approach to the Microbiological Sampling of Surfaces: The Vacuum Probe Sampler.* Albuquerque, Sandia Labs., 1967. (SC-RR-67-114)

FAVERO, M. S. Dual meanings of activation. *Space Newsletter* (Ryde, Aust.) 2(12):163-164, 1967.

FREIFELDER, D., and B. TRUMBO. Matching of single-strand breaks to form double-strand breaks in DNA. *Biopolymers* 7:681-693, 1969.

General Services Admin. *Clean Room and Work Station Requirements, Controlled Environments.* Washington, D.C., GSA, 1966. (Fed. Stand. No. 209a)

GINOZA, W. Radiosensitive molecular weight of single-stranded virus nucleic acids. *Nature* (London) 199:453-456, 1963.

GREEN, R. H. *Environmental Simulation Studies/Molsinl* JPL Planetary Quarantine Program Status Review, June 1968.

HUTCHINSON, F., and E. POLLARD. *In,* Errera M., and A. Forsberg, Eds. *Mechanisms in Radiobiology*, Vol. 1, pp. 71-92. New York, Academic, 1961.

IMSHENETSKIY, A. A., and S. V. LYSENKO. Ultra-high vacuum and microorganisms. *In, Life Science and Space Research*, Vol. 3, pp. 142-148. Amsterdam, North-Holland, 1965.

KAPLAN, H. S., and L. E. MOSES. Biological complexity and radio-sensitivity. *Science* 145:21-25, 1964.

LEA, D. E., and K. M. SMITH. The inactivation of plant viruses by radiation. II. The relation between inactivation dose and size of virus. *Parasitology* (London) 34(2):227-237, 1942.

MAHLER, H. R., and E. H. CORDES. *Biological Chemistry*, 2nd ed., pp. 177-183. New York, Harper & Row, 1966.

MITRA, S., M. D. ENGER, and P. KAESBERG. Physical and chemical properties of RNA from the bacterial virus R 17. *Proc. Natl. Acad. Sci. USA* 50:68-75, 1963.

MORELLI, F. A., F. P. FEHLNER, and C. H. STEMBRIDGE. Effect of ultrahigh vacuum on *Bacillus subtilis* var. *niger. Nature* (London) 196:106-107, 1962.

PHILLIPS, G. B. Back contamination. *Environ. Biol. Med.* 1(2):121-160, 1971.

REYNOLDS, M. C., K. F. LINDELL, and N. LAIBLE. *A Study of the Effectiveness of Thermoradiation Sterilization.* Albuquerque, Sandia Labs., 1970. (SC-RR-70-423)

RUBBO, S. D., and J. F. GARDNER. Efficiency of sterilants in terrestrial and extraterrestrial environments. *In,* Sneath, P. H. A., Ed. *COSPAR Tech. Man. No. 4*, pp. 37-50. Paris, Muray Print, 1968.

SCHACHMAN, H. K., and R. C. WILLIAMS. The physical properties of infective particles. *In,* Burnet, F. M., and W. M. Stanley, Eds. *The Viruses*, Vol. 1, pp. 223-327. New York, Academic, 1959.

SCHUSTER, H. The ribonucleic acids of viruses. *In,* Chargaff, E., and J. N. Davidson, Eds. The *Nucleic Acids: Chemistry and Biology*, Vol. 3, pp. 245-301. New York, Academic, 1960.

SILVERMAN, G. J. Microbial survival in deep space environment. *Environ. Biol. Med.* 1(1):83-97, 1971.

SINSHEIMER, R. L. a single-stranded deoxyribonucleic acid from bacteriophage ϕX 174. *J. Mol. Biol.* 1:43-53, 1959.

STENT, G. S. Mating in the reproduction of bacterial viruses. *In,* Smith, K. M., and M. A. Lauffer, Eds. *Advances in Virus Research*, Vol. 5, pp. 95-149. New York, Academic, 1958.

TAYLOR, D. Effect of planetary trapped radiation belt on microorganisms. Presented at AIBS/NASA semi-annual Spacecraft Sterilization Technology Seminar, San Francisco, Calif., July 1972.

TERZI, M. Comparative analysis of inactivating efficiency of radiation of different organisms. *Nature* (London) 191:461-463, 1961.