

POTENTIAL EFFECTS OF RECENT FINDINGS ON SPACECRAFT STERILIZATION REQUIREMENTS*

S. SCHALKOWSKY**, L. B. HALL† and R. C. KLINE**

Abstract. An important task related to the formulation of planetary quarantine standards is the achievement of an acceptable compromise between (1) the prevention of planetary contamination and (2) the impact of quarantine requirements on the conduct of planetary missions. Such a task is a continuing effort, which must take all pertinent new information into account as it becomes available. This paper provides an analytical framework for the assessment of data which have become available during the past year or which are currently being evolved. In particular, an evaluation is made of the probability of release of viable organisms from the spacecraft as a function of: (1) impact velocity magnitudes and the probability of their occurrence; (2) the degree of equipment fracturing at impact velocities; and (3) the number of viable organisms in spacecraft materials. Work being done to quantify each of three types of contamination, i.e. that on open surfaces, mated surfaces and buried contamination, is described in the context of seeking an approach to spacecraft sterilization that would be most compatible with the implementation of planetary missions. It is concluded that the results of work now in progress on spacecraft-material fracturing, on the estimation of buried contamination loads, and on microbial resistance on mated surfaces, may lead to less severe dry-heat sterilization of planetary spacecraft than had been considered necessary in the past.

1. Introduction

The process of specifying spacecraft-sterilization requirements encompasses numerous factors, many of which contain considerable uncertainty. A suitable analytical model or structure is necessary in order that the various factors be properly weighed and their relative impact on requirements assessed. This paper summarizes the essential aspects of an extended analytical model, beyond that used in the past, to accommodate information which has been developed in the past year, or which is currently being evolved. The various factors currently receiving detailed attention are discussed in this paper and their potential effects on spacecraft-sterilization requirements assessed.

This paper reflects some basic premises currently under consideration in the implementation of planetary quarantine constraints by the National Aeronautics and Space Administration of the U.S.A. In particular, the use of gaseous treatment for spores is viewed as an effective decontaminant, but such treatment is not considered to provide adequate confidence in the destruction of all viable spores present. Similarly, emphasis is placed herein on the evolution of dry-heat sterilization requirements, reflecting an earlier choice of this method over radiation sterilization for spacecraft equipment.

* Work reported herein by Exotech Inc. authors has been supported under contract NASw-1558 with the NASA Office of Planetary Programs and under contract NASw-1666 with the NASA Office of Biosciences.

† National Aeronautics and Space Administration, Washington, D.C., U.S.A.

** Exotech Incorporated, Washington, D.C., U.S.A.

2. Major Considerations in the Formulation of Sterilization Requirements

The degree of risk which should be accepted for planetary contamination has been the subject of discussion in the past. This aspect of the problem is readily summarized in the simple but adequate relationship (*COSPAR Info. Bull.*, 1966; NASA, 1966)

$$P_c = NP(N) + N'P(N'). \quad (1)$$

P_c is the probability that the planet will be contaminated in the course of planetary exploration, and a value agreed upon for this parameter is $P_c = 1 \times 10^{-3}$ (*COSPAR Info. Bull.*, 1966). N and N' are, respectively, the number of landing and non-landing spacecraft which are expected to be flown during unmanned planetary exploration, and $P(N)$ and $P(N')$ are the respective probabilities that any given landing or non-landing flight will cause planetary contamination. Using a total number of flights of $N + N' = 100$ and allowing the contamination probabilities for landing and non-landing missions to be equal, it is readily found that the constraint on any one mission reduces to $P(N) = P(N') \leq 1 \times 10^{-5}$, i.e. the probability that any one planetary spacecraft will contaminate the planet should be $\leq 1 \times 10^{-5}$. In this paper, attention is focused on the requirement $P(N)$ for landing missions since it is for these spacecraft that sterilization procedures become necessary. As demonstrated in connection with planetary fly-by missions, the constraint of 1×10^{-5} can be met for non-landing missions by taking precautionary measures in mission design without having to resort to spacecraft sterilization.

One major area of uncertainty is the probability $P(g)$ of growth and spreading on the planet by microbial contamination of terrestrial origin. Thus, assuming that a viable terrestrial organism has been deposited onto the planet surface, it is necessary to assign a probability that it will grow, spread and bias future biological exploration of the planet. For consistency with the analytical model to be used herein, it is essential to note that this probability refers to a single viable organism released onto the planet surface; the fact that the probability of planetary contamination is increased if more than one viable organism are released is accounted for in the model.

It can be shown that the ratio $P(N)/P(g)$ is no greater than the mean number of viable micro-organisms which can be released onto the planet surface by any one landing spacecraft. This ratio is denoted as $n(r)$. If $P(g) = 10^{-3}$, a value currently considered a conservative assessment of the growth probability on Mars, then $n(r) \leq 10^{-2}$. From the point of view of implementation, $n(r)$ is the controlling planetary quarantine constraint. (The 'mean' number, as used herein to characterize a microbial count, represents the number to be expected, on the average, over repeated trials. For example, $n = 1 \times 10^{-2}$ implies that if a count was repeated 100 times, we would, on the average, expect to find only one organism during one of these counts and no organisms in the other 99 counts.)

The major considerations which enter into the evolution of explicit sterilization requirements from the planetary quarantine constraint on $n(r)$ are summarized in Figure 1. Thus, the landing spacecraft is partitioned into discrete sources of contamination, classified in accordance with actual, physical subassemblies of the spacecraft. The constraint $n(r)$ can therefore be viewed as being distributed amongst all of these subassemblies and the requirement is that the sum of the $n_i(r)$ not exceed the constraint $n(r)$. (The designation $n_i(r)$ refers to the contribution of the i th subassembly.) Within each subassembly a distinction is also made between the following three sources of biological contamination: (1) contamination located on open surfaces; (2) contamination which has been occluded between mated surfaces; and, (3) that which is buried inside spacecraft materials. (In Figure 1 the subscript j denotes the particular source under consideration and the superscripts S , M , B identify the source as being either of the surface, mated or buried type.)

The above classification of sources emphasizes the fact that any one subassembly in the spacecraft can contain, and usually does contain, all three types of contamination sources. The contribution of any one of these sources to the problem can be assessed in terms of the major post-launch and pre-launch factors shown in Figure 1. The major pre-launch factors are (1) the pre-sterilization microbial load at the various spacecraft locations, categorized into surface, mated, or buried types, and (2) microbial resistance to sterilization for the three types of contamination. Referring to the major post-launch factors in Figure 1, all but one of these relate to the probability that viable organisms present in the spacecraft at launch will be released upon arrival at the planet. The first two factors, i.e. spacecraft impact velocities and the probabilities that these impact velocities will occur, are unrelated to the partitioning of the spacecraft into subassemblies or contamination sources. However, the other two release factors are intimately related to this partitioning. Thus, in order to evaluate microbial release from a particular source caused by a crash landing, it is necessary to know to what degree the impact velocity is attenuated at this source. Similarly, the degree of equipment fracturing must be considered in terms of the physical and design characteristics associated with a particular contamination source. The last item noted in the post-launch category is the probability of microbial survival in transit and has to do with the effects of hard vacuum and ultraviolet radiation during flight to the planet.

The major elements of a sterilization specification are shown in the last block of Figure 1. To explicitly define these sterilization controls and procedures, and to do it in a manner which would meet the requisite planetary quarantine constraint, $n(r)$, without unduly constraining mission implementation or unnecessarily degrading engineering and scientific mission success probabilities, it is necessary to quantitatively account for all of the factors shown in Figure 1.

In view of the above, effort is being applied to gain a better understanding of and, where possible, to quantify the major factors in the pre-launch and post-launch categories. In the sections which follow, pertinent aspects of these factors are dis-

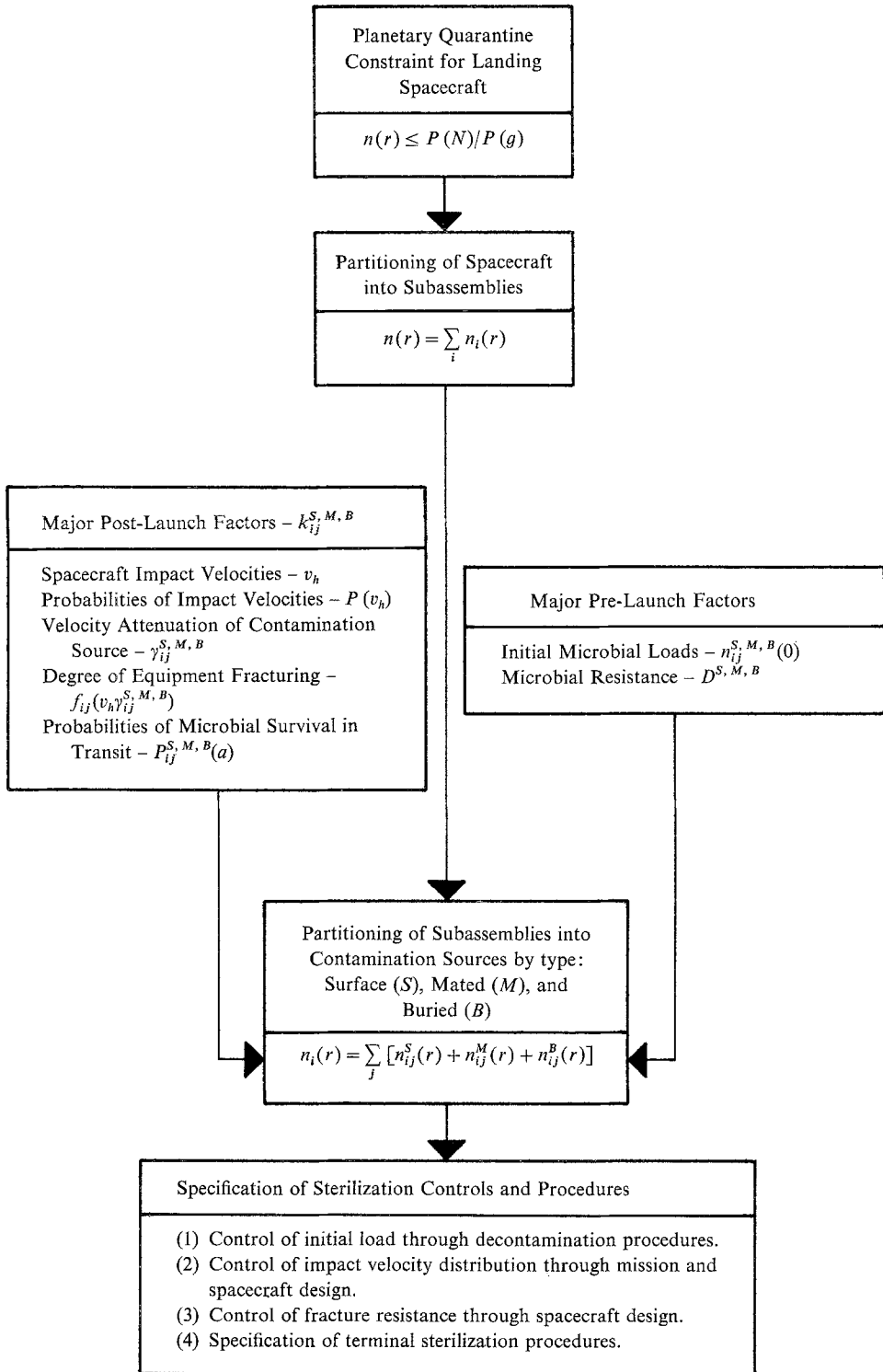


Fig. 1. Major considerations in the specification of sterilization requirements.

cussed as a preliminary step to the consideration of their potential effects on sterilization requirements.

3. Discussion

In discussing the individual post- and pre-launch factors, it will be relevant to establish the degree to which any one of them is either determinable or controllable. The determinability of a factor depends upon how amenable it is to measurement and, also, on the degree of confidence which can be placed upon the values measured or estimated. However, regardless of how well a factor can be determined, it is equally important to establish the degree to which it is controllable, for it is often possible to confine a factor to below a value which would make it a significant influence on the sterilization requirements.

3.1. INITIAL MICROBIAL LOAD - $n_{ij}^{S, M, B} (0)$

Progress made in assessing and quantifying the initial microbial load varies in accordance with the source category considered. Because of the availability of suitable experimental techniques, the accumulation of microbial contamination on open surfaces is most readily assessed. Microbial load on mated surfaces is, of course, the result of the occlusion of what was at a prior stage an open surface. Knowledge of contamination on open surfaces can therefore be transferred, to some degree, also to mated surfaces. However, a direct measurement of mated surface contamination is not readily made. The measurement of microbial loads contained within spacecraft materials is least amenable to effective experimental procedures and reliable data in this category are therefore not available.

Depending upon the size of the spacecraft and controls used in assembly and manufacturing, it is estimated that the microbial load on open surfaces would be in a range between 10^4 and 10^7 . A proportionate range could be applied to mated surfaces. Any estimate of the buried contamination would at this time be largely speculative. However, a reasonable upper bound can be established in terms of microbial concentration per unit volume of material, depending upon the contamination present during manufacturing and heating or other sterilizing factors, which might be natural aspects of the manufacturing or quality assurance processes.

A recent development which may enhance the estimation of buried contamination is associated with the experimental work by PETERSON *et al.* (1968). This work was oriented towards the assessment of microbial release, or exposure, from fractured material, but it now appears feasible to reverse the statistical procedures used and, by fracturing sample spacecraft materials and measuring growth on these fractured surfaces, to obtain an estimate of the concentration of viable contamination in the materials.

The initial load can be controlled or limited during final spacecraft assembly and to a lesser degree during subassembly. Control derives primarily from the use of clean-rooms and/or decontamination procedures. During component manufacture, however, limiting of the contamination load is not too practical.

3.2. MICROBIAL RESISTANCE – $D^{S,M,B}$

The resistance of micro-organisms to dry-heat sterilization has been found to vary considerably depending upon whether the organisms are contained within materials, between mated surfaces, or on open surfaces. In terms of the logarithmic reduction time, i.e. the D -value, or the time required to reduce the population by one decade, the resistance on open surfaces is about 0.3 hours, whereas spores in spacecraft materials have shown resistance as high as 5 hours. On mated surfaces, microbial resistance ranges between 0.3 and about 4.4 hours, depending upon conditions of moisture-vapor transfer at the mated surface and the relative humidity prior to and during sterilization.

It has been well established in the past few years that moisture plays a dominant role in determining the resistance of micro-organisms to heat sterilization (PFLUG, 1967; ANGELOTTI, 1967). Further attention is currently being given to understanding the role of moisture in a way which will permit more effective control over sterilization procedures. This is particularly relevant for mated surfaces, as it would be highly desirable to be able to characterize this type of microbial resistance towards the lower range of the D -values given above and thereby make them nearly equivalent to open surfaces.

3.3. SPACECRAFT IMPACT VELOCITIES – v_h

The velocity of the spacecraft upon arrival on the planet is critical to the consideration of microbial release from the spacecraft. Under nominal soft-landing conditions, there can be microbial release from external surfaces but not from internal surfaces or from the inside of spacecraft materials. In general, it can be assumed that so long as spacecraft landing is at nominal soft-landing velocities, spacecraft equipment will have been designed to operate at these velocities without breakup.

Since hard impact velocities are critical to the estimation of release probabilities, it is not adequate to evaluate them in general terms. Specifically, it is necessary to establish the explicit events for a given planetary mission which would lead to non-nominal landing conditions and to assess the impact velocities, v_h , associated with these events. As mission design progresses, the quantification of these velocities becomes a feasible task.

3.4. PROBABILITIES OF IMPACT VELOCITIES – $P(v_h)$

The explicit events which lead to impact velocities are related to failure modes of particular spacecraft equipments, e.g. deviations from planned midcourse maneuvers, failures in deorbit equipment, or failures in landing-deceleration equipment such as parachutes. The probability that a particular impact velocity will occur is therefore intimately related to the engineering reliability of spacecraft equipment and mission design. The probabilities of various impact velocities will thus be constrained for engineering reasons and the possibility of closer control for quarantine purposes is available, at least in principle.

3.5. ATTENUATION OF SPACECRAFT VELOCITIES - $\gamma_{ij}^{S, M, B}$

As noted in Figure 1, the various release factors must be viewed in the context of discrete spacecraft subassemblies and particular sources of contamination within these subassemblies. It is therefore necessary to ascertain what additional effect may result from the attenuation of spacecraft impact velocity at the source under consideration. In some instances, such as external structural pieces, this may not be too significant a consideration. However, some very fragile subassemblies within a functional element of the spacecraft may have significant velocity attenuation by virtue of the physical path between this element and the point of spacecraft impact. Although a detailed quantification of velocity-attenuation factors may be difficult, it may be possible to estimate them using well-developed theory and empirical knowledge on the shock resistance of structural elements in various configurations. The controllability of this factor can be similarly characterized, i.e. to the extent that techniques are known which will increase impact resistance, they can be utilized in spacecraft design in appropriate circumstances.

3.6. EQUIPMENT FRACTURING - $f_{ij}(v_h \cdot \gamma_{ij}^B)$

In the case of mated and open surfaces, it is assumed that when a critical velocity is reached, contamination from these sources is released. However, in the case of buried contamination it is necessary to identify an additional event before actual release from the inside of materials can occur. Specifically, for any assumed impact velocity, it is necessary to establish the degree to which the material will break up. This parameter is identified herein as the fracture ratio, f , and is given by the ratio of area exposed in the course of impact to the original volume of material under consideration. To complete the characterization of microbial release from materials, it is also necessary to consider a parameter denoted herein as the exposure depth coefficient, λ . This coefficient can, for the present purposes, be viewed as the depth at the exposed surface to which a micro-organism is considered physically free from the material and, therefore, released onto the planet surface.

PETERSON *et al.* (1968) has established experimentally the value of λ to be about 3μ . In these experiments, the value of λ represents, to some degree, the amount of penetration of the nutrient medium into the exposed surface. For the present purpose of considering physical release at impact, it appears reasonable to assume that the value of λ is of the order of the size of the micro-organism, i.e. about 1μ . Considering the uncertainty in other parameters, it is of little consequence at present whether λ is taken to be 1 or 3μ .

Efforts are currently in progress to quantify fracture ratios for typical spacecraft materials, based on information in other areas where experimentation has been carried out. It is also possible to establish upper bounds on the value of the fracture ratio by assuming all of the energy at impact to go into producing fractured areas.*

* Contributions by Dr. William C. Cooley of Exotech Incorporated on obtaining upper bounds of f are gratefully acknowledged.

In general, the fracture ratio, f , would be proportional to the square of the impact velocity. To obtain some feel for the magnitudes of f , consider a solid cube of material about 1 ft on each side. This volume of material would fracture into about 260000 pieces when the fracture ratio is about 1200 1/m. A fracture ratio on the order of 10^6 implies pulverization of the material to micron size and represents a release probability of unity.

3.7 PROBABILITY OF MICROBIAL SURVIVAL IN TRANSIT - $P_{ij}^{S, M, B}(a)$

The effects of ultraviolet radiation on micro-organisms located on the exteriors of the spacecraft, and the effects of hard vacuum on other microbial contamination, have been considered in the past as possible causes of microbial destruction in transit. The effectiveness of ultraviolet radiation is limited by uncertainties on microbial exposure to this radiation. As regards the destructive effects of vacuum in interplanetary space, some initial die-off has been observed in laboratory experimentation but the long-term effects have not been substantiated to make this a major destructive factor (STERN, 1968).

4. Analytical Model

Equation (2) below provides a basic framework for assessing the effect of the various factors discussed above on the development of spacecraft sterilization requirements.

$$\left. \begin{aligned}
 P(N)/P(g) &\geq n(r) \\
 &= \sum_i \sum_j [n_{ij}^S(0) \cdot P^S(s) \cdot k_{ij}^S + n_{ij}^M(0) \cdot P^M(s) \cdot k_{ij}^M + n_{ij}^B(0) P^B(s) \cdot k_{ij}^B] .
 \end{aligned} \right\} \quad (2)$$

The double summation in Equation (2) reflects the need to partition the requirement, $n(r)$, into the various spacecraft subassemblies and to consider within any one subassembly the different contamination sources. The parameter k summarizes all of the post-launch factors which influence the sterilization requirement. To permit a reasonably simple presentation, this parameter is formulated below under the simplifying assumption that the spacecraft will either land at the desired velocity, i.e. a soft landing, or else there will be a single impact velocity denoted by v_h .

$$k_{ij}^S = P_{ij}^S(a) \cdot P_{ij}^S(r) \quad P_{ij}^S(r) = \left\{ \begin{array}{l} 1, \text{ for exterior surfaces} \\ P(v_h), \text{ for interior surfaces if } v_h \geq v_{ij}^S/\gamma_{ij}^S \\ 0, \text{ otherwise} \end{array} \right\} \quad (3)$$

$$k_{ij}^M = P_{ij}^M(a) \cdot P_{ij}^M(r) \quad P_{ij}^M(r) = \left\{ \begin{array}{l} P(v_h), \text{ if } v_h \geq v_{ij}^M/\gamma_{ij}^M \\ 0, \text{ otherwise} \end{array} \right\} \quad (4)$$

$$k_{ij}^B = P_{ij}^B(a) \cdot P_{ij}^B(r) \quad P_{ij}^B(r) = \left\{ \begin{array}{l} \lambda f_{ij}(v_h \cdot \gamma_{ij}^B) \cdot P(v_h), \text{ if } v_h \geq v_{ij}^B/\gamma_{ij}^B \\ 0, \text{ otherwise} \end{array} \right\} \quad (5)$$

where $\lambda = 10^{-6}$ m for f_{ij} in units of 1/m.

The velocities, $v_{ij}^{S, M, B}$, above, represent critical velocities at which the contamination contained at individual sources will be released onto the planet surface. It is to be noted that release from surfaces and mated surfaces is taken to occur only if the

spacecraft impact velocity exceeds this critical velocity, as modified by the attenuation factor for the source considered.

The parameter $P^{S, M, B}(s)$ in Equation (2) denotes the probability that any one micro-organism will survive sterilization of a specified duration. In the case of heat sterilization, $P^{S, M, B}(s)$ could be represented by the corresponding D -values, viz.

$$P^{S, M, B}(s) = 10^{-t/D^{S, M, B}}. \quad (6)$$

The $D^{S, M, B}$, above, are the microbial resistances at a constant sterilization temperature and t is therefore the time required to maintain this temperature in order to achieve a desired value of $P(s)$. In practice, suitable allowances are made for time at transient temperatures in a sterilizing range. For present purposes, t can be viewed as representing the terminal sterilization requirement.

The above model, and extensions thereof which allow for a wider spectrum of impact velocities, is appropriate for operational use in developing specific sterilization procedures and controls. The subject matter of this paper is, however, more readily treated in terms of the simplified version defined below.

5. Potential Effects of Recent Findings

A conservative approach to the implementation of the constraint $n(r)$ would result if the spacecraft impact velocity, v_h , is taken to be larger than the smallest critical velocity, $v_{ij}^{S, M, B}$, at the individual contamination sources. It will also be assumed that microbial destruction in transit will be effective only for external surfaces. It will therefore be convenient to segregate open surfaces into external ones, denoted by the superscript Sx , and internal surfaces, denoted by S . This yields the following expressions for $n(r)$ in terms of total initial contamination on open and mated surfaces and the various factors previously defined:

$$\left. \begin{aligned} n(r) \leq & n^{Sx}(0) \cdot P^{Sx}(a) 10^{-t/D^S} \\ & + P(v_h) [n^S(0) \cdot 10^{-t/D^S} + n^M(0) \cdot 10^{-t/D^M} + \lambda \cdot 10^{-t/D^B} \sum_i \sum_j n_{ij}^B(0) f(v_h \cdot \gamma_{ij}^B)] \cdot \end{aligned} \right\} \quad (7)$$

It is evident from Equation (7) that the terms for each source category, i.e. for open surfaces, mated surfaces, and buried contamination, must separately be less than the quarantine constraint, $n(r)$. Furthermore, that term in Equation (7) which is largest will necessarily dominate the specification of the sterilization time t . The principal questions, therefore, relate to which of these source categories represents the dominant term and whether the dominant term yields the smallest terminal sterilization time. A corollary question is whether a preferred term could be made dominant. Figure 1 indicates a number of controls which might be made a part of the specification of sterilization procedures for the above purpose. For example, design constraints may be imposed on spacecraft-impact velocities and/or the probabilities of their occurrence. Similarly, some latitude may be available in altering critical velocities of components which may contain large contamination loads, or to improve the velocity attenuation at these sources through appropriate design proce-

dures. Another control is that of minimizing the contamination load through the use of clean-rooms and related procedures. Some, or all, of these, may be useful. However, to justify their use, it must be ascertained that they are contributing to the reduction of a dominant term in Equation (7).

Until recently, a conservative estimate was made of the probability of release of buried contamination. In terms of the parameters defined herein, a probability of release of unity is equivalent to a fracture ratio of about 10^6 , which implies pulverization of the entire spacecraft. This is clearly not a reasonable estimate of conditions which are likely to occur. Although work on fracture ratios of typical spacecraft materials is still in progress, it is evident that the fracture ratio will be significantly lower than that implied in earlier estimates. In any event, the probability of release must be less than unity by virtue of the fact that the probability of non-nominal landing velocities is less than unity.

Earlier conservative estimates of microbial release of buried contamination, combined with the known higher resistance of such contamination to heat sterilization, have made buried contamination the dominant term and, necessarily, led to a relatively stringent terminal sterilization requirement. Referring to the terms in the parentheses of Equation (7), it is likely that work now in progress will show the product $\lambda \sum_i \sum_j n_{ij}^B(0) f(v_n \cdot \gamma_{ij}^B)$ to be smaller than $n^M(0)$. This would imply a shift towards mated contamination as a basis for defining sterilization requirements. However, to benefit from such a shift in any significant way, D^M would have to be significantly smaller than D^B . For, as noted earlier, current work sets the value of D^M between 0.3 and 4.4 hours and the upper value is very close to microbial resistance for buried contamination, upon which requirements have been based to date. There is thus a need to gain a better understanding of both the effects of equilibrium humidity and pressure at the mated surfaces during assembly and sterilization. This may then produce a value of D^M closer to 0.3 hours, and lessen the ultimate sterilization requirements.

It is also evident from Equation (7) that even very low fracture ratios and low microbial load for buried contamination could not move sterilization procedures to the point where only gaseous or other non-thermal (or radiation) treatment could be used. To permit consideration of the latter approaches, a significant change would have to occur in the value of $n(r)$, i.e. either in $P(N)$ or $P(g)$. For unless the value of $n(r)$ is on the order of unity, or larger, each of the terms on the right side of Equation (7) must be significantly less than unity. This implies sterilizing methods which can be relied upon to destroy all spores present with a high degree of confidence.

6. Summary and Conclusions

Work currently in progress is focused on the following areas:

(1) the degree of spacecraft equipment fracturing at spacecraft impact velocities, both in materials and at equipment interfaces, so as to obtain more realistic estimates of probabilities of microbial release;

(2) microbial resistance to heat sterilization at mated surfaces and the physical conditions which will determine its magnitude;

(3) estimation of microbial contamination buried in spacecraft material.

The above work, combined with suitable controls over mission and spacecraft design procedures, may lead to less stringent terminal heat sterilization requirements than had been considered necessary in the past. A determination of the specific values to be specified for terminal heat sterilization must, however, await the more detailed quantification of the various parameters discussed herein; it will at all times depend upon the values selected for the quarantine goal, namely, the probability assigned to the risk of any one landing mission contaminating the planet, and the probability estimated for any one viable terrestrial micro-organism spreading and growing on the planet surface.

References

- ANGELOTTI, R.: 1967, 'Protective Mechanisms Affecting Dry Heat Sterilization'. COSPAR Symposium on Sterilization Techniques, London, July.
- Committee on Space Research: 1966, Paris. COSPAR Resolution 26.5. *COSPAR Information Bull.* 33. October.
- National Aeronautics and Space Admin: 1966, A Note on COSPAR Resolution 26.5. *NASA Position Paper*, May.
- PETERSON, N. J., CORNELL, R. G., and PULEO, J. R.: 1968, 'Release of Microbial Contamination from Fractured Solids', *Space Life Sci.* 1, 531.
- PELUG, I. J.: 1967, 'Dry Heat Sterilization: Rates of Destruction and Temperature Coefficients'. COSPAR Symposium on Sterilization Techniques, London, July.
- STERN, J. A.: 1968, Personal Communication, April.