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A CRITIQUE OF CURRENT SPACECRAFT STERILIZATION STANDARDS*

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Introduction

The following summarizes some of the comments made by the author to the NASA Bio-Science Subcommittee during the December 16, 1965 meeting in Atlanta, Georgia. Further elaboration on these comments is also provided when appropriate.

This discussion centers around the analysis and numerical estimates reported by C. Sagan and S. Coleman in the May 1965 issue of Aeronautics and Astronautics in the article titled "Spacecraft Sterilization Standards and Contamination of Mars" (p. 22). As noted by the editors of the above journal (page 23), this article "served as the basis for international discussion at the COSPAR meeting held in Florence, Italy, in May 1964 . . . . Using the analytical framework presented in the article, but adopting slightly different numerical values, the working group concluded that the 'probability that a single viable organism be aboard any vehicle intended for planetary landing must be less than 1×10^{-4},' and that 'the probability of accidental planetary impact by an unsterilized flyby or orbiter must be less than 3×10^{-5} during the interval terminating at the end of the initial period of planetary exploration by landing vehicles (approximately one decade).' The report of the study group was accepted by the Consultative Group and by the Executive Council of COSPAR."

Summary of Sagan-Coleman Analysis

The basic relationship which summarizes the Sagan-Coleman analysis is (see equation 10 of the referenced article):

$$\ln P^{-1} = \frac{\sigma N P_m}{\chi P_e \cdot P_t \; P_l} + nP_i \qquad (1)$$

* Work supported under contract NASW-1340 with the NASA Office of Bioscience.

where

p - probability that Mars will not be contaminated before N experiments are successfully completed

σ - probability of one viable micro-organism on surface of Mars due to a single lander

N - desired number of successfully completed experiments in unmanned Mars exploration program

P_m - probability that one organism deposited on the surface of Mars will survive, grow and spread, thus leading to planetary contamination

χ - mean number of experiments per lander

P_e - mean probability that an experiment will work as designed

P_t - probability that the lander vehicle will perform its engineering functions after it is landed on the planetary surface

P_l - probability of finding experimental conditions on Mars (e.g., kind of life) compatible with experiment design

n - number of fly-bys and orbiters

P_i - probability of accidental impact by a fly-by or orbiter.

Best estimate values are adopted by Sagan and Coleman in order to illustrate the magnitude of σ and P_i as follows

$p \approx 0.999$

$N \approx 1200$

$\chi \approx 20$

$P_m \approx 10^{-2}$

$P_e \cdot P_l \approx 10^{-1}$

$P_t \approx 0.9$

$n \approx 30$

To permit the assignment of specific values to P_i and σ, it is assumed that the division of contamination risks between all landers, on the one hand, and all orbiters and flybys on the other is equally divided between the two. Calculations are then reported by Sagan and Coleman to lead to

$$\sigma \approx 2 \times 10^{-4}$$

$$P_i \approx 4 \times 10^{-5}$$

COSPAR Resolution

To permit a comparison between the COSPAR values and those of Sagan and Coleman, it is first of all necessary to reconcile the fact that the former deals with a probability of a single viable microorganism being aboard the landing vehicle, whereas in the latter σ refers to the probability of a microorganism being released on the surface of Mars. The two can be correlated by the relationship

$$\sigma = P_N \cdot P_R \qquad (2)$$

where P_N - probability of one viable microorganism aboard the lander

P_R - mean probability that one microorganism, if present, will be released from the lander and deposited on the Martian surface

If P_R is assumed to be unity, the COSPAR definition would be identical to that of Sagan and Coleman. As regards numerical values, there is also little difference between the two, viz.

	σ	P_i
COSPAR	1×10^{-4}	3.5×10^{-5}
Sagan and Coleman	$2 \times 10^{-4} (0.75 \times 10^{-4})$	4×10^{-5} (2×10^{-5})

The numbers in parenthesis are corrected values, as calculated by the author. The difference stems largely from the use by Sagan and Coleman of logarithms to the base 10 rather than the base e called for by equation (1).

Although the author is not aware of an explicit statement from COSPAR regarding the values used for p, N, χ P_m, P_e, P_l, P_t and n in arriving at the recommended values for σ and P_i, it can be inferred from the closeness to the Sagan and Coleman values of σ and P_i that approximately the same estimates were used.

Modification of Sagan-Coleman Analysis

It will be convenient to rearrange equation 1 as follows:

The term $\ln p^{-1}$ can be replaced by p_c, where p_c denotes the probability that Mars will be contaminated before N experiments are successfully completed. Since

$$p_c = 1-p$$

$$\ln p^{-1} = \ln \frac{1}{1-p_c} = -\ln(1-p_c) = p_c + \frac{1}{2} p_c^2 + \frac{1}{3} p_c^3 + \text{----}$$

For small values of p_c, e.g., $p_c = 10^{-3}$,

$$\ln p^{-1} \approx p_c \tag{3}$$

If we denote by M^L the number of lander launches needed to provide N successful experiments and by R^L the mean probability that a launch will result in successfully landing on Mars, we can write

$$M^L \cdot R^L = \frac{N/\chi}{P_e \cdot P_t \cdot P_l} \tag{4}$$

Using equations 2, 3, and 4, equation 1 can be written as

$$P_c = M^L \cdot R^L \cdot P_N \cdot P_R \cdot P_m + nP_i \qquad (5)$$

If the analysis of probabilities of contamination is to have any practical significance, it is essential that P_N, the probability of a single viable microorganism aboard a lander, be given a realistic meaning. To date, spacecraft sterilization practice has been based on the extrapolation of logarithmic kill rates due to dry heat, assuming a single species. P_N is then obtained from

$$P_N = N_o \cdot 10^{-t/D} \qquad (6)$$

where N_o - initial population of microorganisms on the lander (prior to the application of dry-heat)

t - length of time dry heat is applied at a particular fixed temperature

D - time it takes to reduce a single-species population by a factor of 10 at a fixed temperature

Equation 5 and 6 can be combined, subject to the constraint that $N_o \cdot 10^{-t/D} < 1$. The resulting expression, equation 7 below, should be the basis for evaluating contamination hazards.

$$P_c = M^L \cdot R^L \cdot N_o \cdot 10^{-t/D} \cdot P_R \cdot P_m + nP_i \qquad (7)$$

For the purpose of this discussion we assume (1) a single species microbial population on the lander (2) dry-heat sterilization at a constant temperature and (3) that the extrapolation to values of $P_N \ll 1$ is valid. The last assumption as well as the basic logarithmic kill rate expression are subject to question. Also, additional refinements could be introduced to account for mixed populations with variable dry-heat resistances and to allow for heat-up and cool-down times during sterilization. However, these points can, and should be considered independently, i.e., without complicating the present discussion.

As is evident from equation 5 or 7, the approach to be discussed here for evaluating planetary contamination hazards consists of allocating risks between to independent events: (1) contamination due to a sterilized lander, $p_c(L)$, and (2) contamination due to accidental impact of an unsterilized orbiter or flyby, $p_c(B)$. Thus

$$p_c = p_c(L) + p_c(B) \tag{8}$$

The definition of $p_c(B)$ could be enlarged to encompass all other sources of accidental contamination, e.g., recontamination of a sterilized lander by rocket exhaust from the unsterilized bus, contamination by meteoroid impact on an unsterilized bus or orbiter in the vicinity of Mars, etc. $p_c(L)$, however, deals strictly with the residual hazard after dry-heat sterilization of the lander.

Discussion

Equation 4, defining the number of Mars lander launches needed to obtain the desired number of successful experiments, leads to the following result if parameter values adopted by Sagan and Coleman are used:

$$M^L \cdot R^L = \frac{N/\chi}{P_e \cdot P_t \cdot P_l} = \frac{1200/20}{(0.9)(0.1)} = 666$$

If we now assume an average reliability figure for successfully landing a vehicle on Mars of $R^L = 0.9$, we obtain

$$M^L = \frac{666}{0.9} = 740$$

This is clearly an unrealistically high number of lander missions to be expected, * particularly if the time period under consideration is "approximately one decade", as noted in the COSPAR statement. Furthermore, this figure is

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* Similar calculations were carried out by P. M. Sprey of Grumman and were brought to our attention by Dr. L. Slote, also of Grumman.

inconsistent with the value of n=30 assumed by Sagan and Coleman for the number of orbiters and flybys during the same time period. For, as a first approximation, it would be more reasonable to assume that the number of orbiters and/or flybys will equal the number of landers, i.e., every lander mission must have a bus to bring it to Mars thus providing an opportunity for the accidental impact of an unsterilized vehicle. (Some buses will carry two landers, but there will also be some purely orbiting or flyby missions, i.e., without landers). The large difference noted above therefore casts some doubt as to whether contamination hazards are suitably apportioned between $p_c(L)$ and $p_c(B)$.

A calculation similar to the above, but using the values adopted by COSPAR of $P_N = 10^{-4}$, $P_i = 3 \times 10^{-5}$, can be made using equation 5. In this case we do not assume anything as to the desired number of successful experiments but seek to infer the values of M^L and n which would result if $p_c \approx 10^{-3}$, $p_R \approx 1$ and $P_m \approx 10^{-2}$. The latter are the estimates adopted by Sagan and Coleman. To make this calculation we also assume an equal distribution of hazards between $p_c(L)$ and $p_c(B)$, i.e.,

$$M^L \cdot R^L \cdot P_N \cdot P_R \cdot P_m = nP_i = \frac{1}{2} p_c \qquad (9)$$

Setting $R^L \approx 0.9$, we now obtain

$$M^L = \frac{(0.5)\ 10^{-3}}{(0.9)\ 10^{-4}\ \ 10^{-2}} \approx 555$$

and

$$n = \frac{(0.5)\ 10^{-3}}{3 \times 10^{-5}} \approx 17$$

The inconsistancy in the Sagan-Coleman analysis thus seems to have been carried over into the COSPAR standards for P_N and P_i. To bring the values of M^L and n into better agreement we might reasonably assume $M^L \approx n \approx 30$ for the decade under consideration. To be more conservative, we should set $R^L \approx 1$. The resulting values of P_N and P_i are then

$$P_N = \frac{(0.5)\ 10^{-3}}{(30)\ 10^{-2}} \approx 2 \times 10^{-3}$$

$$P_i = \frac{(0.5)\ 10^{-3}}{30} \approx 2 \times 10^{-5}$$

The above calculations are intended to indicate that current standards may well have been based upon unrealistic estimates of the extent and nature of the Mars exploration program in the immediate future. It is, however, not intended to suggest that presently accepted standards be modified on the basis of the above alone. Indeed, it is the principal contention of this author that insofar as spacecraft sterilization is concerned, the formal adoption of any number for P_N without regard as to how it will be implemented, is of little practical value and should therefore not be done.

To amplify the above comments, reference is made to equation 7 in which P_N has been replacedby its current operational equivalent, i.e., $P_N = N_o\ 10^{-t/D}$. Thus, whereas before P_N was looked upon as a requirement to be met, its operational equivalent contains two unknowns, N_o and D, which rather than being specified must be estimated or evaluated. The only item that can be specified is t, the spacecraft sterilization time, and the magnitude which will be selected for it will depend upon the values adopted for all of the parameters in the equation. The latter process should be a mutually consistent one but there is good reason to believe that this may not now be the case.

For the present purposes, we restrict ourselves to the part of equation 7 which deals with the residual contamination hazard after heat-steri lization of landers, i.e.,

$$p_c(L) = M^L \cdot R^L \cdot N_o \cdot 10^{-t/D} \cdot P_R \cdot P_m \qquad (10)$$

Currently, heat sterilization requirements are based upon heat resistance of heterogeneous mesophilic bacterial spores in soil. Deferring for the moment questions as to the validity of the assumed logarithmic rate of population reduction and the extrapolation of this data to numbers much smaller than 1*, at least the following two items deserve further consideration:

(a) The selected D value is based upon laboratory tests in which survival times are established in terms of particular recovery media, e.g., soil extract broth, glucose culture, TGYE, thioglycollate, etc., each producing different survival times. The particular D value selected should therefore be related to that part of P_m which deals with the probability of growth on the Martian surface. Specifically, if P_m is estimated as being about 10^{-2}, it must be recognized that this represents the probability that a spore which has been subjected to heat-sterilization will (1) survive the Martian freeze-thaw cycle and ultra-violet environment (after release from the lander onto the planetary surface) and (2) find substances on the Martian surface equivalent to the laboratory culture medium on the basis of which the D value has been selected, e.g., thioglycollate. In addition, P_m incorporates the probability that contamination started in one locale will spread to other parts of the planet.

(b) Regardless of the particular value chosen for P_m, P_R or p_c, it is generally recognized that the process of selection is one of rough estimation and that the

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* Exotech Inc. is currently engaged in a study dealing with these and related items under contract NASW-1340 from the Office of Bioscience of NASA Headquarters.

most appropriate approach for the present is that of assuming the worst case. It is therefore difficult to find justification for approaching N_o, the initial microbial population on the lander, through bio-assay of actual spacecraft equipment. Certainly, an accurate count of N_o, even if possible, would not greatly increase the accuracy of estimating planetary contamination hazards since it is only one number in a product of many, each of which contains large uncertainties. The merit of bio-assay of spacecraft equipment as an integral part of the operational sterilization program is therefore subject to serious question.

An alternative consistent with the estimation of other contamination parameters would be to assume the worst case for N_o. For example, if the basis for estimation is the heat resistance of the heterogeneous microbial population in garden soil, we might assume that the entire lander consists of nothing but such soil. The spore count in soil is about 2×10^6 per gram* and we might therefore estimate N_o from

$$N_o \approx W \times 10^9 \qquad (11)$$

where W = weight of lander in pounds

To complete the illustration, assume a lander weight of 2,000 lbs. We will also use the following parameter estimates

$$p_c \approx 10^{-3} \quad ; \; p_c(L) = \frac{1}{2} p_c$$

$$M^L \approx 30$$

$$R^L \approx 1$$

$$P_R \approx 1$$

$$P_m \approx 10^{-2}$$

Using equations 10 and 11 we obtain $t \approx 15D$

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* Dry garden soil, heat shocked in water suspension at 80°C for 10 minutes - see C. W. Bruch, M. G. Koesterer and M. K. Bruch in Developments in Industrial Microbiology, Vol. 4, 1963 (AIBS).

To fully specify the sterilization time t, it would still be necessary to select a D value consistent with $P_m \approx 10^{-2}$. However, regardless of this choice, and noting the very conservative estimates for the other parameters, 15D values would appear to be the maximum length of sterilization to be expected. Furthermore, this maximum requirement does not depend upon bio-assay, special clean-rooms or ETO decontamination, i.e., it could readily be applied to a vehicle assembled by conventional techniques. This contrasts sharply with current requirements and procedures for heat sterilization of landing vehicles.

Concluding Remarks

The formal distinction between the sterilization "standard" P_N (or σ) and sterilization requirements defined in terms of N_o and t (based on a choice of D), appears to have led to a wide divergence between sterilization practices and the purposes which they are intended to serve. This distinction should therefore be eliminated (except where it serves calculation convenience) and the subject of spacecraft sterilization requirements be re-examined in detail.

The comments contained herein are intended to highlight the need for a re-examination rather than offer specific alternatives. To accomplish the latter will require a broader and more detailed evaluation than that provided here. It is not unreasonable to expect, however, that such a re-examination may lead to significant changes in heat sterilization requirements and simplification of related procedures, without detriment to planetary contamination probabilities. Benefits which are likely to be derived from the enhancement of lander reliability and from reduced program costs can also not be overlooked.