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EXOTECH
INCORPORATED

ANALYTICAL TECHNIQUES IN PLANETARY QUARANTINE

FINAL REPORT
Contract NASw-1734

for

Headquarters
National Aeronautics & Space Administration
Planetary Quarantine Office

by

EXOTECH INCORPORATED
525 School Street, S.W
Washington, D.C. 20024

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TRSR 70-13

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I. INTRODUCTION AND SUMMARY

I. INTRODUCTION AND SUMMARY

Contract NASw-1734 was initiated 23 April 1968. It specified analysis and planning activities to support NASA's Planetary Quarantine (PQ) Office in four areas, viz..

- Review and Interpretation of Planetary Quarantine Requirements
- Analysis of Microbial Survival
- Development and Application of Pertinent Analytical Techniques
- Implementation of a Lunar Chemical Contamination Inventory

The results of the work in these areas is reported herein Where preliminary findings have already been presented in interim reports, these are referenced. Copies of these interim documents are appended in those cases where their content is felt necessary to support the material presented herein.

A WORK ELEMENTS

A listing follows of the work elements involved in the four tasks enumerated above.

(1) Review and Interpretation of Planetary Quarantine Requirements

- Development of an approach to the justification of modified PQ requirements.
- Review of PQ requirements for proposed Planetary Explorer Missions to Mars and Venus.
- Review of Mars Mariner '69 PQ Plan
- Review of Viking Project PQ Provisions document.
- Development of plans for the review of Viking PQ related documents.

(2) Analysis of Microbial Survival

- Survival curve analysis

  Study of physical processes in microbial resistance to sterilization

(3) Development and Analysis of Pertinent Analytical Techniques

- Analytical estimation of buried contamination.
- Study of microbial release through material break up and erosion.

(4) Planetary Chemical Contamination

- Planning for the implementation of a Chemical contamination inventory.

B. DELIVERED PRODUCTS

Among the products delivered under this contract are

- Exotech Inc., Systems Res. Div.: An Analytical Basis for Assaying Buried Biological Contamination. Appendix C. Report no. TRSR-036. Jan. 1969.
- Exotech Inc., Systems Res. Div.: Implementation of a Chemical Contaminant Inventory for Lunar Missions. Appendix E. Report TRSR 70-07. Dec. 1969.
- Barrett, M. J.· Investigations into a Diffusion Model of Dry Heat Sterilization. Appendix B. Report no. TRSR-041, Systems Res. Div., Exotech Inc., May 5, 1969.
- DeGraff, E.. Review of JPL Report 605-87, Mariner Mars 1969 PQ Plan. Systems Res. Div., Exotech Inc., Memo., Aug. 21, 1968.
- NASA Headquarters Review of Viking PQ Plans. A Document Providing Background Information for Reviewers of Viking Plans.
- Space Bioscience Research material dated Nov. 5, 1969 for Code SB Document on Applications.
- Proposed Planetary Chemical Contamination Requirements statement, Feb 1969.
- Guidelines for Review of JPL-Martin Bioburden Accumulation Model, Nov. 13, 1969.
- Exotech Inc., Systems Res. Div.: Monthly Letter Status Reports as Required by the Contract.

C. PRESENTED PAPERS

- "Potential Effects of Recent Findings on Spacecraft Sterilization Requirements", presented at the 11th Plenary Meeting of COSPAR, Tokyo, Japan, May 14, 1968.
- Report on "Development of Analytical Techniques in Planetary Quarantine", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, June 10, 11, 12, 1968

- "Effects of Mated D-Values on Terminal Sterilization Cycle", presented to Subcommittee 1A of PQAC, University of Minnesota, July 25 - 26, 1968.
- "Stochastic Diffusion Model for Microbial Survival in Heat Sterilization", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969.
- "Systems Approach to Compliance with PQ Requirements", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969.
- "Estimation of the Mean Concentration of Microorganisms Buried in Spacecraft Materials", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969.
- "Tradeoff Studies in Heat Sterilization" presented to a committee of the Space Science Board at Stanford University on April 19, 1969.
- "Consequences of Stochastic Diffusion of Moisture in Microbes", presented at Semi-Annual Planetary Quarantine Seminar, Las Vegas, September 24 - 25, 1969.
- "Application of a Systems Model for Spacecraft Sterilization", presented at Semi-Annual Planetary Quarantine Seminar, Las Vegas, September 24 - 25, 1969.

D. OTHER MEETINGS ATTENDED

Additional meetings attended include:

- PQAC, NASA/Hdgts., October 1968.
- Microbiological Assay Standardization Meeting, NASA/Hdqts., December 13, 1968.
- Planetary Explorer PQ Meeting, NASA/Hdqts , January 29, 1969.
- Taft Health Center, Cincinnati, Ohio, April 22, 1969 - Review of Experimental Data on Microbial Die Off.
- Lunar Sample Analysis Symposium, 157th National Meeting American Chemical Society, Minneapolis, Minnesota, April 13 - 18, 1969.
- Viking Planetary Quarantine Review Meeting, Martin Co., Denver, Colorado, October 22, 1969.

II. RESULTS

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Because extensive material has already been reported in interim documents and papers, those results will not be duplicated in this final report. Where previous findings are felt necessary for the reader, they are provided in summarized form or appended.

The material presented herein extends and complements this previous work. It is reported in the sequence of the four tasks enumerated in the previous section.

A. REVIEW AND INTERPRETATION OF PQ REQUIREMENTS

The pre-contract status of planetary quarantine standards, their formulation and their impact upon spacecraft programs was summarized in a paper[1] presented at the 11th Plenary Meeting of COSPAR on May 11, 1968, in Tokyo, Japan.

Although the work summarized at COSPAR was supported under earlier contracts (NASw-1558 and NASw-1666), a copy of the paper is attached (Appendix A) because it adds to the prospective of the work reported herein. The formulation of planetary quarantine standards was shown to be a continuing effort requiring a compromise between the assurance of the prevention of planetary contamination and the minimization of the impact of quarantine requirement on planetary missions. It was suggested that further work in several areas could lead to less severe sterilization requirements for planetary spacecraft than had been considered necessary in the past. Such areas for further research include tasks undertaken in the study reported herein. Of specific interest in this regard are.

- Estimation of buried contamination.
- Analysis of release of buried contamination.
- Microbial resistance to sterilization processes.

Work in these areas is reported in Tasks B and C.

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[1] Exotech Incorporated, Potential Effects of Recent Findings on Spacecraft Sterilization Requirements, May 1968, also Space Life Sciences 1 (4) March (1969) 520-530.

The focus of activity in this initial task of contract NASw-1734 was in the application of planetary quarantine requirements. This is typified in the review of PQ constraints conducted in January 1969 for two proposed Planetary Explorer missions to orbit Venus and Mars. Probabilities of contamination for the two missions were computed based upon COSPAR constraints and estimates made by NASA's Planetary Program Office of the total number of different missions to be conducted within the quarantine period by all nations. The characteristics of the proposed Planetary Explorer missions were reviewed in the light of these PQ requirements. An analysis[2] performed by Bird Engineering Research Associates Inc. of the probability of contamination for the two missions was reviewed for accuracy and completeness

Spacecraft project plans and provisions for compliance with PQ requirements were studied. In particular, the Planetary Quarantine Plan proposed for Mars Mariner 1969 was assessed.[3] The Viking project PQ Provisions document[4] was reviewed for compliance with PQ standards. In addition, plans were developed for the systematic review of further Viking project PQ related documents including:

- The PQ Plan
- The Microbiological Assay and Monitoring Plan
- The Sterilization Plan

Methodology for the development and evaluation of feasible alternative approaches for compliance with PQ constraints was presented in the Semi-Annual Planetary Quarantine Seminar at Cape Kennedy in February 1969.

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[2]Letter report 24 February 1969, Bird Engineering Research Associates Inc. to GSFC, Code 724 "Preliminary Report on Planetary Explorer Contamination Problem."

[3]Exotech letter 21 August 1968, subject Review of JPL Report 605-87.

[4]Langley Research Center document, "Viking Planetary Quarantine Provisions", No. M73-109-0, February 20, 1969.

B. ANALYSIS OF MICROBIAL SURVIVAL

Knowledge of the life and death processes of microorganisms is essential to the development of realistic PQ requirements and NASA's Planetary Quarantine Office has directed several research efforts in a total program with the objective of expanding this knowledge. Exotech's efforts in this program involved a study of microbial resistance to sterilization. An earlier report[5] had suggested the advantages of log-normal over the simpler logarithmic model to describe the decay of viable microorganisms when subjected to heat sterilization. This report recommended that further analytical work be complemented by physical modeling and be closely related to measured laboratory parameters.

The exceptional resistance of microbial spores to sterilization by heat had long been observed in laboratory tests. The lack of a cohesive theory to explain this behavior, however, represented a weakness in the development of a defensible basis for the formulation of sterilization requirements. Exotech attempted under this contract to form an empirical framework from fragmentary theories and hypotheses so as to relate the effectiveness of environmental factors on dry heat sterilization as practiced in the NASA planetary quarantine program.

Earlier work reviewed included the "water activity" theory of Murrell and Scott[6] who observed experimentally the existence of an optimum water content in the spore that maximizes spore resistances to heat destruction, water and heat effect measurements by Angelotti[7], and more recent research

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[5] Schalkowsky & Wiederkehr "Estimation of Microbial Survival in Heat Sterilization" COSPAR Technique Manual No. 4, November 1968.

[6] W. G. Murrell and W. J. Scott, "The Heat Resistance of Bacterial Spores at Various Water Activities", J. Gen Microbiol. 43, 411-425 (1966).

[7] R. Angelotti, "Protective Mechanisms Affecting Dry-Heat Sterilization", COSPAR Technique Manual Series, Manual No. 4, November 1968.

of water intake and release by Campbell and coworkers[8]. Various mechanisms have been considered in an effort to explain this often-observed non-logarithmic heat destruction characteristic of spores in terms of their water content and its changes.

In the model under investigation the heat resistance of spores is attributed to the partial dehydration of some unidentified but vital protein in the cytoplasm. During sporulation, chemical binding of water to this protein is inhibited by the chelating agent, calcium dipicolinate. This action frees nonessential water molecules, which can then be squeezed from the cell by the surrounding cortex that appears to contract as the spore forms. During wet heat sterilization, water diffuses through the outer layers of the spore and interchanges with the chelating agent, thus, increasing the susceptability of the vital protein to denaturation, resulting in nonviable spores. The large differences between resistances of species may be due to the variable efficiency of the outer layers in inhibiting water diffusion. The great difference in heat resistance of B. subtilis and B. stearothermophilus in aqueous solutions, which essentially disappears in dry heat sterilization, can be attributed to this diffusion effect.

A time dependence of the spore's water activity can be predicted on the basis of a diffusion mechanism whereby loosely bound water migrates from one molecule in the microbe to another, with a mobility determined by the temperature and the existing gradient, according to well-known physical laws of diffusion.

A technical report was presented at the February 1969 Semi-Annual Planetary Quarantine Seminar at Cape Kennedy and early results were submitted in Exotech report TRSR-041, "Investigations into a Diffusion Model of Dry Heat Sterilization", May 1969, attached as Appendix B Further results on this effort were reported at the Semi-Annual Planetary Quarantine Seminar in Las Vegas in September 1969. At that time the existence of two

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[8] Private communication, J. E. Campbell, PHS, Cincinnati, Ohio.

distinct bonding sites for water in B. subtilis was demonstrated from data[9] supplied by Campbell. A sample of the evidence presented for this conclusion is given in Figure 1, which is a replot of the experimental measurements, demonstrating that the rate of water release can be described by the sum of two exponentials. This supports the theoretical framework of the diffusion approach, but further work would be necessary to identify and locate the two sites within the spore.

The implications of this work to the quarantine program are significant. First, it promises an analytical basis for the observed deffi-ciencies in the simple logarithmic description of the microorganism die-off curve. The existence of a significant "tail off" in this curve can perhaps be quantitatively defined and predicted, thus, providing a defensible basis for the design of realistic sterilization cycles. This work also suggests the advisability of monitoring or perhaps controlling the humidity levels during assembly of spacecraft to modify the contaminants present for greatest susceptibility to subsequent sterilization conditions. Perhaps a low temperature preconditioning cycle during which the water activity is equilibrated at some sensitive value could precede high temperature sterilization to make it more effective. Finally, water content of such materials as lucite and epoxy might be controlled during manufacture to make their buried contaminants more susceptible to sterilization.

C. DEVELOPMENT AND APPLICATION OF PERTINENT ANALYTICAL TECHNIQUES

The need for improved estimation of the amount of buried contamination and of its probability of release was indicated in earlier work (see page 2) as worthy of further study. The difficulties in detecting and enumerating buried contamination derive from their inaccessibility. To overcome this, the contaminated material may be physically shattered or chemically dissolved and the resultant released microorganisms then counted. In both cases, however, there are serious questions of the validity of the yield since die-off can occur in the release processes.

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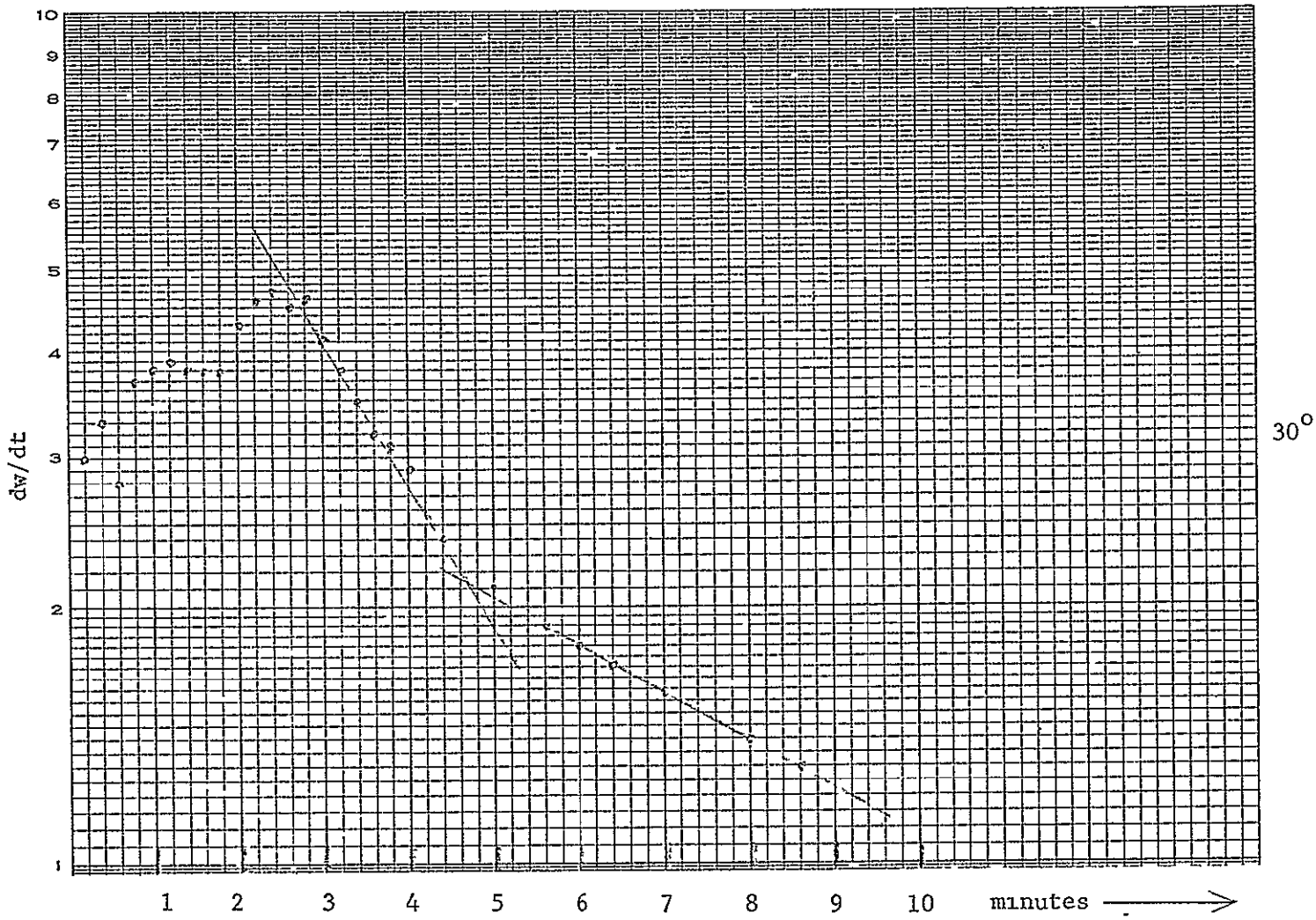
[9] Private communication, J. E. Campbell, PHS, Cincinnati, Ohio.

FIGURE 1
RATE OF WATER RELEASE AT 30°



Rate (mg/sec) at which water is released by a sample of B. subtilis spores in a current of dry air at 30°C. (Datus points, provided by Dr. J. E. Campbell of the Taft Health Center, Cincinnati, Ohio, have been corrected for background water release. Straight lines indicate that the sum of two exponentials would reproduce measurements after an initial build-up period.)

A statistical assay technique originally suggested by Mr. L. Hall, NASA Planetary Quarantine Officer, was further developed by Exotech and a protocol[10] proposed for its application (See Appendix C) The statistical assay technique does not require that all microorganisms contained in the material be recovered but produces an upper bound estimate based upon assay results of new surface exposed by minor fracturing which introduces minimum lethality.

The procedure requires the controlled fracture of representative samples of a material whose buried loading is of interest. Each sample is tested for biological contamination on the totality of new surfaces exposed as a result of the fracturing process. The basic datum or observation consists of the proportion of samples which yield contamination upon culturing. Conventional statistical techniques, combined with an analytical expression relating the mean concentration of organisms buried within the material and the observed datum, produce an upper bound estimate for the unknown mean concentration, expressed to any prescribed level of confidence. In principle, the "confidence level" of the resulting estimate is directly related to the sample size and the amount of surface area exposed by fracture; as the sample size and exposed area increase, the difference between the estimate and the unknown mean load tends to decrease.

The procedure can be very useful in the development of more realistic spacecraft sterilization specifications where the sterilization cycle is sensitive to the estimated number of buried contaminants. In particular, a significant decrease in the severity of the cycle may be possible if realistic estimates of the buried bioloads can be developed. (These benefits are not restricted to spacecraft applications but could also be extended into sterilization processes in the food and drug and other spin off areas.)

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[10] Exotech Incorporated, TRSR-036, "An Analytical Basis for Assaying Buried Biological Contamination", January 1969.

A preliminary analysis[11] of the probability of microbial release of buried contamination considered two release mechanisms; viz., break up in hard impact and aeolian erosion. Further, it formulated analytic expressions for the probability of release as a function of pertinent parameters and identified areas of uncertainty.

Specifically, the fracture ratio, i.e., the ratio of newly exposed area to the initial volume of material, upon which the non-nominal landing probability of release is especially sensitive, was one area of study. The results of impact tests[12] conducted by the Boeing Aircraft Company were reviewed and a method was devised for accounting for impact die off. The ratio of the viable spores recovered from the fragments to the initial load seeded into the pellet is a product of the release depth, the fracture ratio and the degree of impact die off. Values for release depth can confidently[13] be assigned in the 1 to 3 micron range. Fracture ratios can be determined by physical measurement of the recovered fragments. The accuracy of this measurement varies with the number and size of the fragments and has yet to be quantitatively bounded.

When values for release depth and fracture ratio are assigned, the recovered lethality ratio is then a direct measurement of the impact die off. This method of analysis will be applied when additional impact data becomes available.

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[11] Exotech Incorporated, TRSR 70-03, "Development and Application of a System Model for Spacecraft Sterilization", August 1969.

[12] S. J. Fraser, "Survival and Release of Viable Microorganisms After a Hard Impact", Boeing Company report #D2-114143-1, May 27, 1968

[13] N. J. Peterson, R. G. Cornell, and J. R. Puleo: "Release of Microbial Contamination from Fractured Solids." Paper presented at 11th Planetary Meeting of COSPAR, Tokyo, Japan, May 1968.

Further study of theoretical fracturing may provide an insight into fracturing mechanisms. An upper bound to the area exposed during impact can be calculated from the kinetic energy at impact, knowing the energy required for a unit area of fracture. The materials of interest are mostly noncrystalline, buried viable spores are inconceivable in metal structures. Measurements in polymeric materials give 0.5 in.-lb/in^2 (about 10^5 erg/cm^2) for energy per unit area of fracture.[14] This estimate may be useful for preliminary upper-bound calculations of fracture associated with hard impact landings. Further work remains in this area.

Microbial release due to aeolian erosion was also studied and is reported in Appendix D[15]. Although a model has been developed, conclusions as to whether a release factor significantly less than unity for Mars can be invoked will require an agreed upon estimate of Martian meteorology. It is well known that astronomers have interpreted shifting haze that occasionally obscures the planet's features as being due to large sandstorms. Sand or dust is, of course, a major erosion agent, and simulations of the erosion effect of postulated sandstorms have been reported[16]. Analysis of the photography of the planet's surface by Mariner 6 and 7 tends to support the sandstorm hypothesis· the scarcity of small craters in the size distribution can be accounted for by an erosion process.

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[14] M. L. Williams, "The Fracture of Viscoelastic Material" in Fracture of Solids, D.C. Drucker and J. J. Gilman (ed,), Interscience (1963).

[15] M. J. Barrett and J. L. Woodall: The Release of Buried Microbial Contamination by Aeolian Erosion Exotech Report No. TRSR 70-14.

[16] G. Dyhouse, "Simulated Martian Sand and Dust Storms and Effects on Spacecraft Coatings", ASTM/IES/AIAA Second Space Simulation Conference (Sept. 1967). Am. Soc. Testing Materials, 1967, Philadelphia, Pennsylvania.

A second area of uncertainty that deserves investigation is the possibility of spore destruction during the erosion process. The dimensions of a spore, typically on the order of a micron, are considerably smaller than those of the average sand particle encountered on Earth. Those dust particles in the micron range are generally more dominant in the stratosphere, and particle size can be loosely correlated with altitude since smaller size permits particles to be carried higher and farther by the wind. Presumably, the same mechanisms apply on Mars, and a lander in a Martian dust storm would be pelted by particles considerably larger than a spore. The erosion model to be applied, therefore, is akin to the use of boulders in chipping away seeds. One suspects a significant amount of lethality in this process.

Further study should concentrate on areas most conducive to permitting the unit value of probability of release to be reduced thereby alleviating sterilization cycle requirements. Analysis should be conducted to identify the areas of uncertainty where additional knowledge will produce the maximum benefit. Each factor should then be reviewed to permit selection of those most achievable within time and resource constraints. Effort should be expended only so long as a payoff in terms of further relaxation of the probability of release will result.

C. CHEMICAL CONTAMINATION

A common prerequisite for space exploration is the absence of contamination by terrestrial material in the area being explored. In the search for biological life, this concern has led to the establishment of rigorous planetary quarantine standards. The danger, however, exists in all science fields where experiment objectives can be compromised by the undesired introduction of unknown quantities of "foreign" matter.

In previous work[17], Exotech studied the problems of unintentional contamination of lunar samples to be returned to Earth for analysis.

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[17]Exotech Incorporated, TRSR 68-029, "Planning Study for an Organic Constituents Inventory Program", May 1968.

Adopting a philosophy that absolute purity cannot be guaranteed and that some contamination will be introduced by astronauts, vehicle ejecta and the capsule environment, an approach was developed which attempts to identify and quantify the types and levels of expected contamination. This is achieved by establishing an inventory of organic materials contained in Lunar landing spacecraft as part of an information system which can identify regions of high contamination risk and the probable contaminating materials.

The study undertaken in this contract defines the initial step in the implementation of the above approach. It considers the detailed procedures and tasks necessary to collect, evaluate, store and disseminate data which will serve anticipated needs of lunar sample investigators, consistent with the requirement that costs associated with implementation and operation of the inventory be compatible with known needs for this information. The primary tasks pertinent to this effort involved. (1) determining the availability of lunar mission vehicle documentation and the means for collecting it in a form suitable for future evaluation, (2) the collection and utilization of spacecraft trajectory parameters, landing sites, and dispersion patterns for crash and soft landings, and (3) evaluation of the compatibility of required data inputs with an existing Planetary Quarantine information system. The final report[18] of this effort is appended.

Should further implementation be warranted, future efforts should include the following

- Retention of documentation identifying types and quantities of chemical materials in lunar mission spacecraft landers.

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[18] Exotech Incorporated, TRSR 70-07, "Implementation of a Chemical Contaminant Inventory for Lunar Missions," December 1969

- Designation of a Federal Records Center to receive these documents and organization of a suitable filing system
- Preparation of a contamination risk model of the lunar surface based on the best available estimates of spacecraft landing sites and the particle dispersion associated with the mass and velocity characteristics of hard and soft landers.
- Dissemination among lunar sample investigators, and others associated with the planning of lunar scientific exploration, of information concerning the documentation to be kept in storage.

With the advent of landing missions to the planets of the solar system, similar attention is warranted to the question of chemical contamination by terrestrial material. The study should be extended to consider the acquisition of documentation and material samples from planetary flight missions. Information requirements of current and potential experimenting scientists who will analyze planetary materials should be collected. Information system designs which can serve these needs for data on material contamination should be formulated.

III. CONCLUSIONS AND RECOMMENDATIONS

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Planetary Quarantine requirements, policies and constraints should be reviewed in the light of past experience and new knowledge to insure that maximum possible relaxation of sterilization specifications is realized.

The implication on planetary quarantine requirements for approved flight projects of recommendations made by the Space Science Board at its December 11, 1969 meeting should be evaluated.

2. Flight project quarantine plans should be evaluated for their responsiveness to NASA PQ requirements and compatibility with accepted practices for their implementation.

3. Past planetary flights should be evaluated to develop realistic levels for the probability of contamination for future missions

4. The probability that microorganisms contained on landing spacecraft will be released in a viable state on a planetary surface should be further studied. The effect of fracture upon impact and aeolian erosion during the period of biological exploration and the survivability of microorganisms in the course of the above release mechanisms should be considered.

5. Analytical models should be developed to facilitate the implementation of planetary quarantine requirements on flight projects through the utilization of new laboratory data or related technological progress in the following areas:

   (a) Estimation of microbial contamination in spacecraft environments.

   (b) Dry heat resistance of microorganisms on open surfaces, mated surfaces and buried contamination in spacecraft equipment. Microbial die off in dry heat sterilization should be further analyzed with emphasis on the role of moisture content.

(c) Thermal dynamics in heat sterilization of spacecraft equipment.

(d) System tradeoff model encompassing all relevant factors prior to, during, and after the application of heat sterilization.

6. The requirements for a planetary chemical inventory should be studied Potential information requirements of scientists who will perform analyses of planetary materials to be collected during planetary flight missions should be identified, other material information systems should be investigated for their applicability.

7. A quarantine document system should be designed specifically oriented to serve the management of planetary quarantine on flight missions.

APPENDIX A

Potential Effects of Recent Findings on Spacecraft Sterilization Requirements

POTENTIAL EFFECTS OF RECENT FINDINGS ON SPACECRAFT STERILIZATION REQUIREMENTS

S. Schalkowsky[2]*, L. B Hall[1] and R. C. Kline[2]*

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 has become available during the past year or which is 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

*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.

(1) National Aeronautics and Space Administration, Washington, D. C.
(2) Exotech Incorporated, Washington, D. C.

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 United States. 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.

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) and NASA (1966),'

$$P_c = N\,P(N) + N'\,P(N') \qquad (1)$$

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 microorganisms 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 ith 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

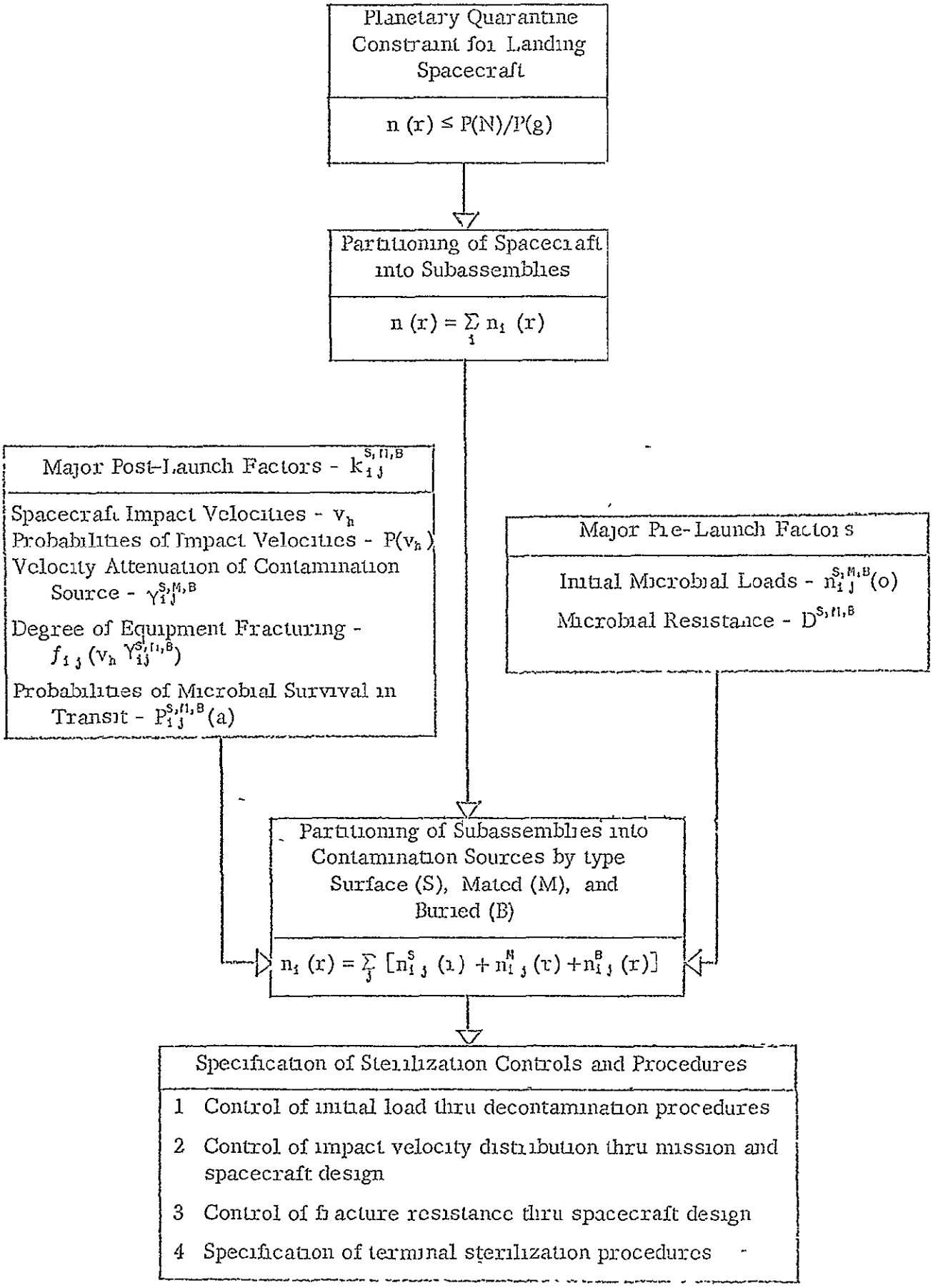

Figure 1 Major Considerations in the Specification of Sterilization Requirements

two release factors are intimately related to this partitioning. Thus, in order to evaluate microbial release caused by a crash landing from a particular source, 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 discussed 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 - $n_{ij}^{s,fl,s}(o)$

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 - $D^{S,H,B}$

The resistance of microorganisms 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 microorganisms to heat sterilization (Pflug, 1967 and 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 - $\gamma_{ij}^{s,fl,B}$

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 - $f_{ij}(v_h \cdot \gamma_{i\,j}^{B})$

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 noted herein by 'Peterson et al. (1968)' 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 microorganism 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 3 microns. 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 microorganism, i. e. about 1 μ. Considering the uncertainty in other parameters, it is of little consequence at present whether λ is taken to be 1 or 3 microns.

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.*

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* 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 260, 000 pieces when the fracture ratio is about 1, 200 ι/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 microorganisms 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.

$$P(N)/P(g) \geq n(r) =$$

$$= \sum_i \sum_j \left[n_{ij}^S(o) \cdot P^S(s) \cdot k_{ij}^S + n_{ij}^M(o) \cdot P^M(s) \cdot k_{ij}^M + n_{ij}^B(o) P^B(s) \cdot k_{ij}^B \right] \quad (2)$$

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.

$$k^S_{ij} = P^S_{ij}(a) \quad P^S_{ij}(r) \qquad P^S_{ij}(r) = \begin{cases} 1, & \text{for exterior surfaces} \\ P(v_h), & \text{for interior surfaces if } v_n \geq v^S_{ij}/\gamma^S_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{3}$$

$$k^M_{ij} = P^M_{ij}(a) \quad P^I_{ij}(r) \qquad P^M_{ij}(r) = \begin{cases} P(v_h), & \text{if } v_h \geq v^M_{ij}/\gamma^M_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{4}$$

$$k^B_{ij} = P^J_{ij}(a) \cdot P^B_{ij}(r) \qquad P^B_{ij}(r) = \begin{cases} \lambda f_{ij}(v_h \quad \gamma^B_{ij}) \cdot P(v_h), & \text{if } v_h \geq v^B_{ij}/\gamma^B_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{5}$$

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

The velocities, $v^{S,M,B}_{ij}$, 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 microorganism 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.

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

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

$$n(r) \leq n^{sx}(o) \cdot P^{sx}(a)\ 10^{-t/D^{S}} +$$

$$+ P(v_h)\left[n^{s}(o) \cdot 10^{-t/D^{S}} + n^{M}(o)\ 10^{-t/D^{M}} + \lambda \cdot 10^{-t/D^{B}} \sum_{i} \sum_{j} n^{B}{}_{ij}(o)\, f(v_h \cdot \gamma^{B}_{ij}) \right] \tag{7}$$

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 corollory 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 procedures. 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 parenthesis of equation 7, it is likely that work now in progress will show the product $\lambda \sum_i \sum_j n^B_{ij}(o)\, f(v_h \quad \gamma^B_{ij})$ to be smaller than $n^M(o)$. 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; and, (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 microorganism spreading and growing on the planet surface.

REFERENCES

Committee on Space Research, Paris. COSPAR Resolution 26.5. COSPAR Information Bull., No. 33 Oct. 1966.

National Aeronautics and Space Admin. A Note on COSPAR Resolution 26 5 NASA Position Paper, May 1966.

Angelotti, R. Protective Mechanisms Affecting Dry Heat Sterilization. COSPAR Symposium on Sterilization Techniques, London, England. July 1967.

Pflug, I. J. Dry Heat Sterilization Rates of Destruction and Temperature Coefficients. COSPAR Symposium on Sterilization Techniques, London, England. July 1967.

Peterson, N. J., R. G. Cornell, and J. R. Puleo. Release of Microbial Contamination from Fractured Solids. Presented at the 11th Plenary Meeting of COSPAR, Tokyo, Japan, May 9-21, 1968. [Submitted to Space Life Sciences for publication]

Stern, J. A. (California Inst. of Tech., Jet Propulsion Lab.) Personal Communication. April 1968.

APPENDIX B

Investigations into a Diffusion Model of Dry Heat Sterilization

INVESTIGATIONS INTO A DIFFUSION MODEL OF DRY HEAT STERILIZATION

Interim Report

Contract NASw-1734
for
National Aeronautics and Space Administration
Office of Biosciences

Prepared by

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May 5, 1969

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TRSR-041

ABSTRACT

The analytical model described in this study formalizes the hypothesis that dry heat inactivation of microorganisms is closely related to the water content of the spore and its micro-environment. Experimental data are examined relative to this model and it appears to be valid. This model is aimed at overcoming the well known deficiencies of the logarithm model.

INTRODUCTION

Several recent investigations have related environmental conditions other than temperature to microbial spore survival under heat sterilization. Murrell and Scott, Angelotti, and Reid have shown strong dependency of dry heat death rates on ambient relative humidity and on water content of the spore*. Hunnell and Ordall have found that heat causes spores to exude calcium and dipicolinic acid (DPA) prior to death, while Alderton, Thompson, and Snell show that spores in aqueous suspension have improved heat resistance when calcium ions are present.

In addition to these correlations with environmental conditions during sterilization, spore survival shows correlations with environmental conditions that prevailed when the spore formed. Vinter shows heat resistance is affected by calcium and cystine availability during sporulation; Murrell and Warth show correlations of heat resistance with five different substances found in the spore.

These investigations show the importance of environment on heat sterilization characteristics. A simple chemical reaction does not appear to be the complete mechanism for spore destruction. Rather, a sequence of transport of chemicals, notably water, occurs and modulates the rate at which chemical reactions destroy the viability of the spore.

In this report we present a diffusion-denaturation model of spore heat resistance that attempts to correlate with the water effects observed, and to provide a basis for determining the efficiency of proposed dry-heat sterilization plans. Such a model is immediately useful in a dry-heat sterilization program, and also offers promise of eventual understanding of the remarkable resistance of spores to heat.

*References are listed at the end of this report.

DIFFUSION MODEL

A model to predict the water content of a spore as a function of time, temperature and initial water concentration within the spore has been developed. The assumptions made are that heat deactivation in spores is due to protein denaturation and that the rate of this reaction is controlled by the water content of the spore core.

A spore is composed of an outer coat (cortex plus spore coats) and a central core (cytoplasm) which is the dormant micro-organism. The cortex protects the core from physical damage, chemical contamination and rapid wetting by the environment. The cortex is mainly composed of mucopeptide polymers (Warth et al, 1963) with the chains twisted and coiled or interwoven (Mayall and Robinow 1957). The coat is mainly protein with a high cystine disulphide bond content (Vinter 1961).

The heat resistance of spores is assumed to rest in the ability to control the amount of water internal to the spore. A contractile cortex system (Lewis, Snell and Burr 1960) would provide a mechanism to dehydrate the cytoplasm and maintain it in this state. Chemical variation in the mucopeptide of the cortex and in the amount of Cu ++ or Ca-DPA (Young 1959) binding to the mucopeptide which may cause the contraction, could result in differences in the degree of contraction and therefore in the final water content of the spore, resulting in marked difference in heat resistance.

In the development of this diffusion model we have assumed a simplified spore structure composed of an outer coat of negligible thickness at a radius surrounding a spherical, homogeneous one.

Water transport thru the spore can be described by the well known diffusion equation

$$D\nabla^2 C = \frac{dC}{dt} \qquad (1)$$

where C is the concentration of water/cm^3, as a function of position and time. D is a diffusion coefficient depending on the medium.

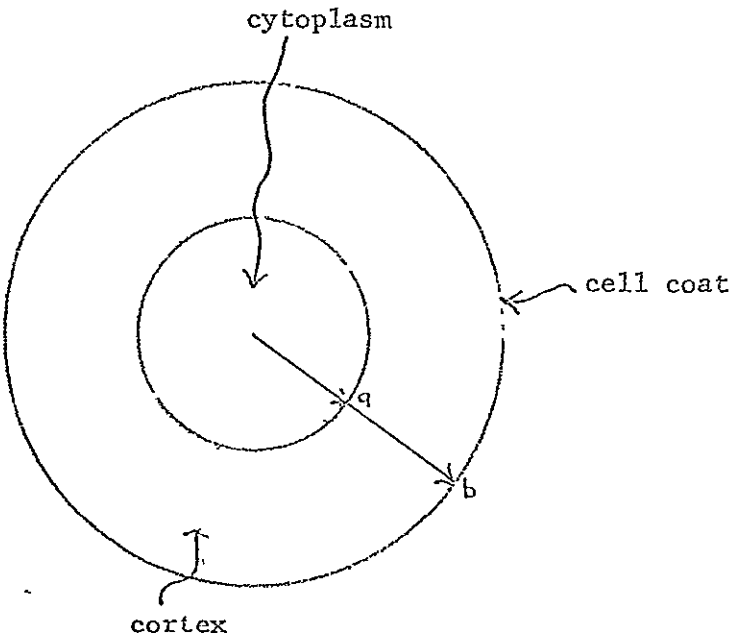

Figure 1. Model of Spore

For a simple example, assume the initial water content in a spherical spore is distributed radially according to

$$rC(r) = \frac{b}{\pi}\left[\sin\frac{\pi r}{b} - \tfrac{1}{2}\sin\frac{2\pi r}{b}\right] \qquad (2)$$

where C(r) is the water concentration at a distance r from the center of the spore, and b is the radius of the spore (see Fig 1). This expression, as seen in Figure 2, corresponds to a distribution that is peaked in the outer portion of the spore, and can be considered as approximating the case where a spore contains more water than an optimum amount. This excess water is stored in the cortex.

Applying the diffusion equation to this initial distribution results in the time variation shown in Figure 3. Water diffuses inward to the cytoplasm, and the spore gradually loses water to the medium. As a result, the initially-dry central region reaches a peak concentration of water that occurs around a time t given by

$$\frac{D\pi^2 t}{b^2} = 1.5 \qquad (3)$$

where D is the diffusion coefficient of the system. This simple model assumes D to be independent of position. As a result, it approximates the situation when the spore is buried in an appropriate medium, since normally one would expect the diffusion coefficient of water outside a spore to differ from D inside the spore.

Data have been reported by Angelotti (1966) for the thermal sterilization of spores in lucite. These data (Fig 4) indicate the death rate to be greatest at about 1.5 hours after commencing to heat the spores at 125°C. Using this value for t, in the above equation, we arrive at an estimate for the diffusion coefficient at 125°C.

$$D = 1.2.10^{-6} \ (cm^2/sec) \qquad (4)$$

This estimate is in reasonable agreement with values for diffusion of protein molecules in water (Clark, p. 138). Our example requires the opposite water molecule diffusion in protein, which has an unknown diffusion coefficient,

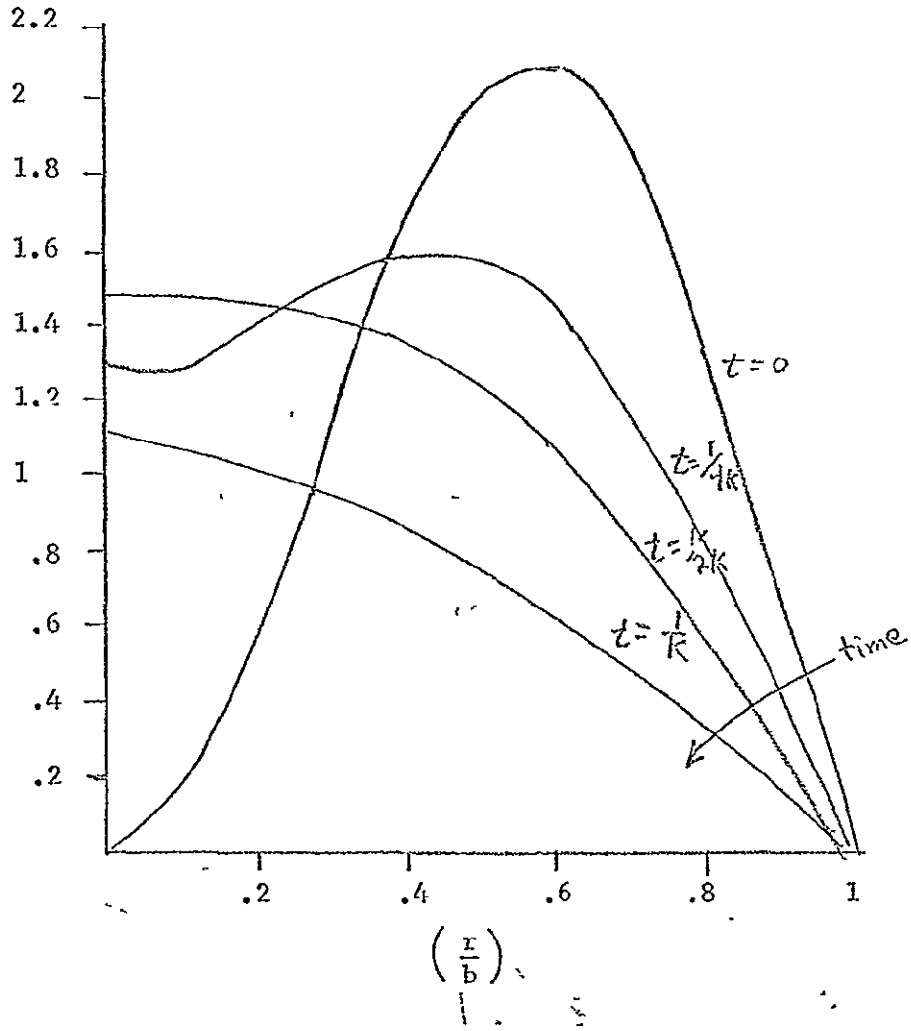

Figure 2. Water Distribution in the Spore

For $t = o$, $\quad rG = \frac{b}{\pi}\left[\sin\frac{\pi r}{b} - \frac{1}{2}\sin\frac{2\pi r}{b}\right]$

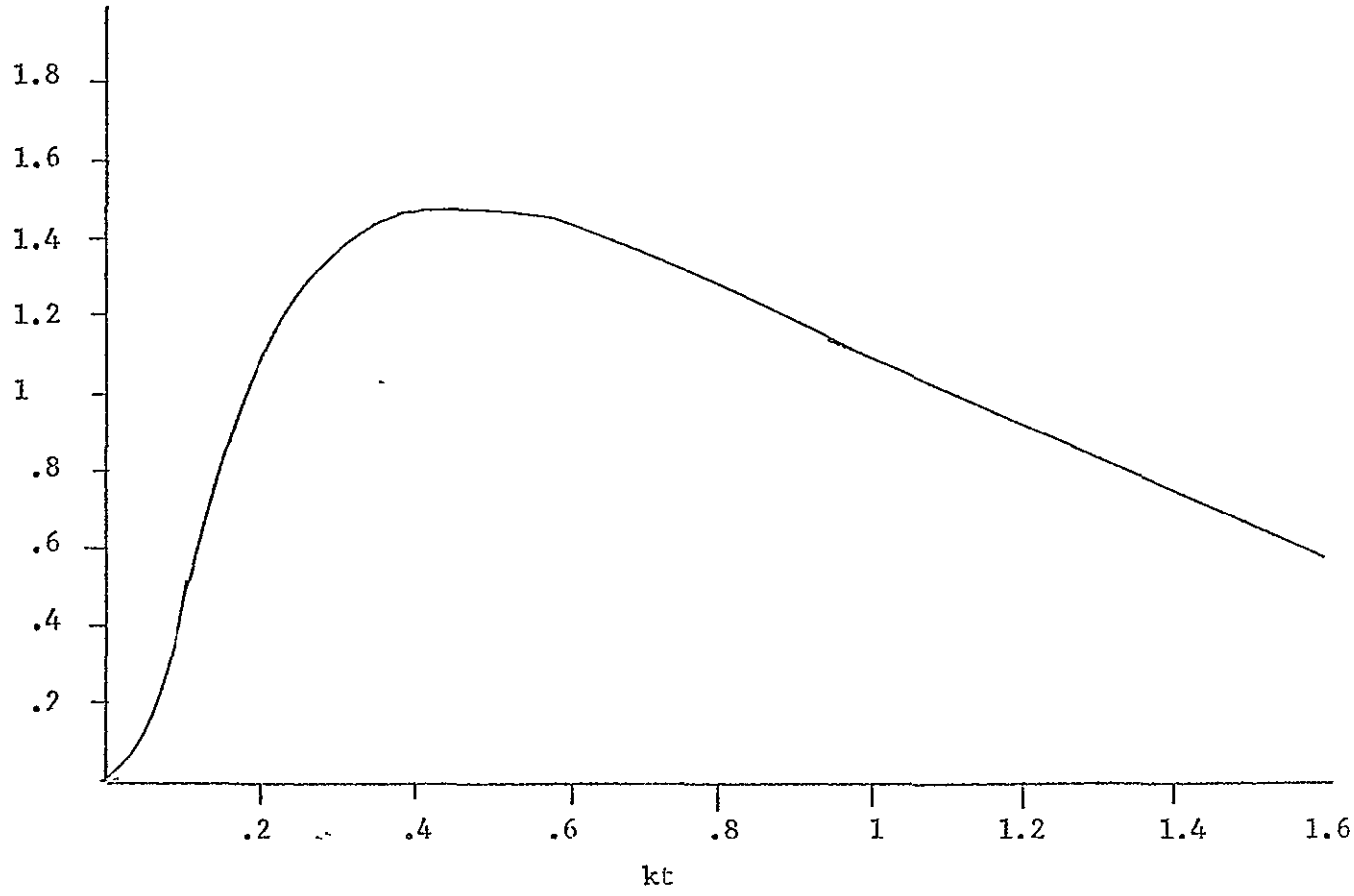

Figure 3. Concentration of Water at center of spore, as a function of heating time.

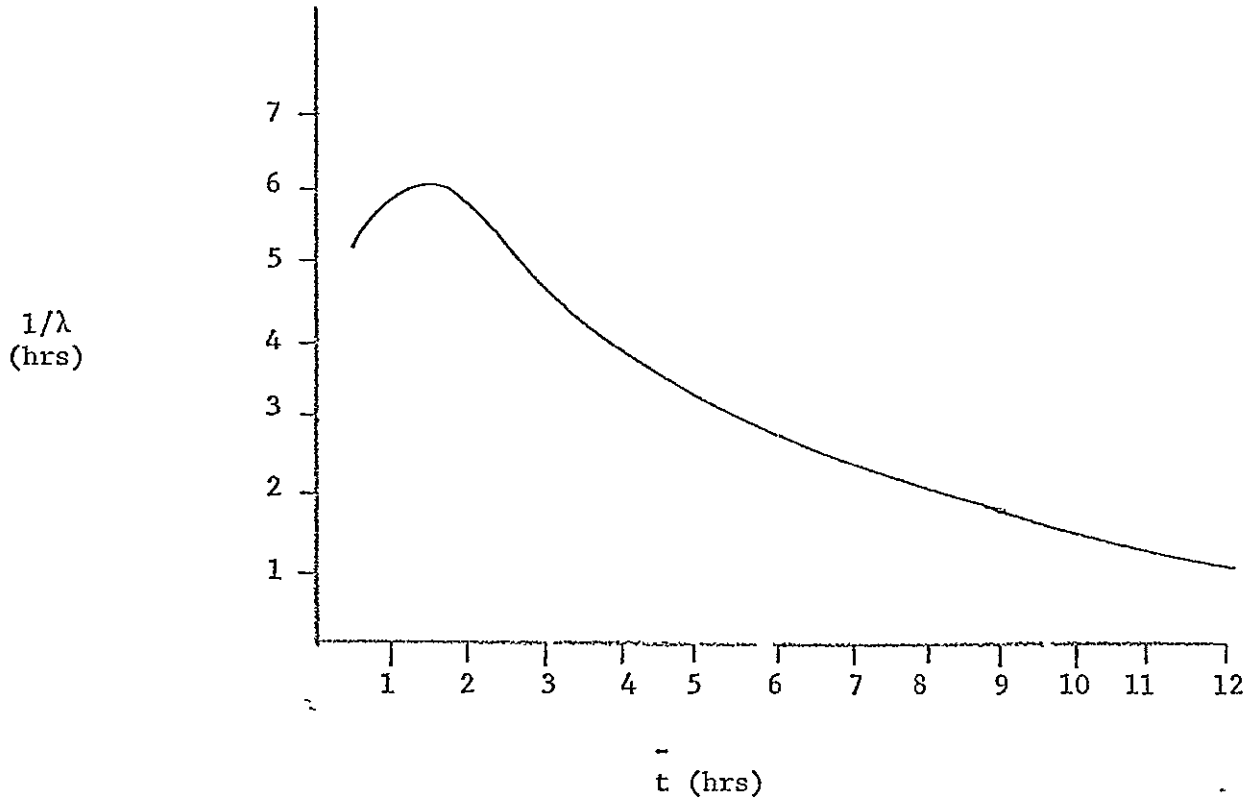

Figure 4. Apparent change in resistance of B. globigii in lucite after times $\bar{t}$ in hours at 125° C

and further assumes this coefficient does not differ greatly from water diffusion in lucite.

One method of evaluating the diffusion coefficient, which is necessary if a diffusion model is to be applied to dry sterilization, is to look at wet sterilization curves. Such curves are shown in Figure 5 for four temperatures. In wet heat, the spores should absorb water to some maximum. Then, the denaturation of proteins in the spore, with this water present, results in a straight logarithmic curve for survivors, as a function of time of heating.

Such a description fits the curves shown. The knee of each curve represents the time at which the cytoplasms of the spores are in equilibrium with the external water. For a purely exponential buildup of water, this occurs about when three relaxation times have passed, according to diffusion kinetics. The diffusion coefficient calculated by this method can be fitted to a formula

$$D = D_o e^{H/RT}, \text{ where } \begin{cases} H = 60 \text{ K cal} \\ R = 1.98 \\ T = \text{temperature } (^{o}K) \\ D_o = \text{Constant} \end{cases} \qquad (5)$$

Results of the calculations are shown in Figure 6, together with the diffusion coefficient calculated as Eqn. 5. The two sets of experiments seem to be in fair agreement, and indicate initial success in applying a diffusion model to the data.

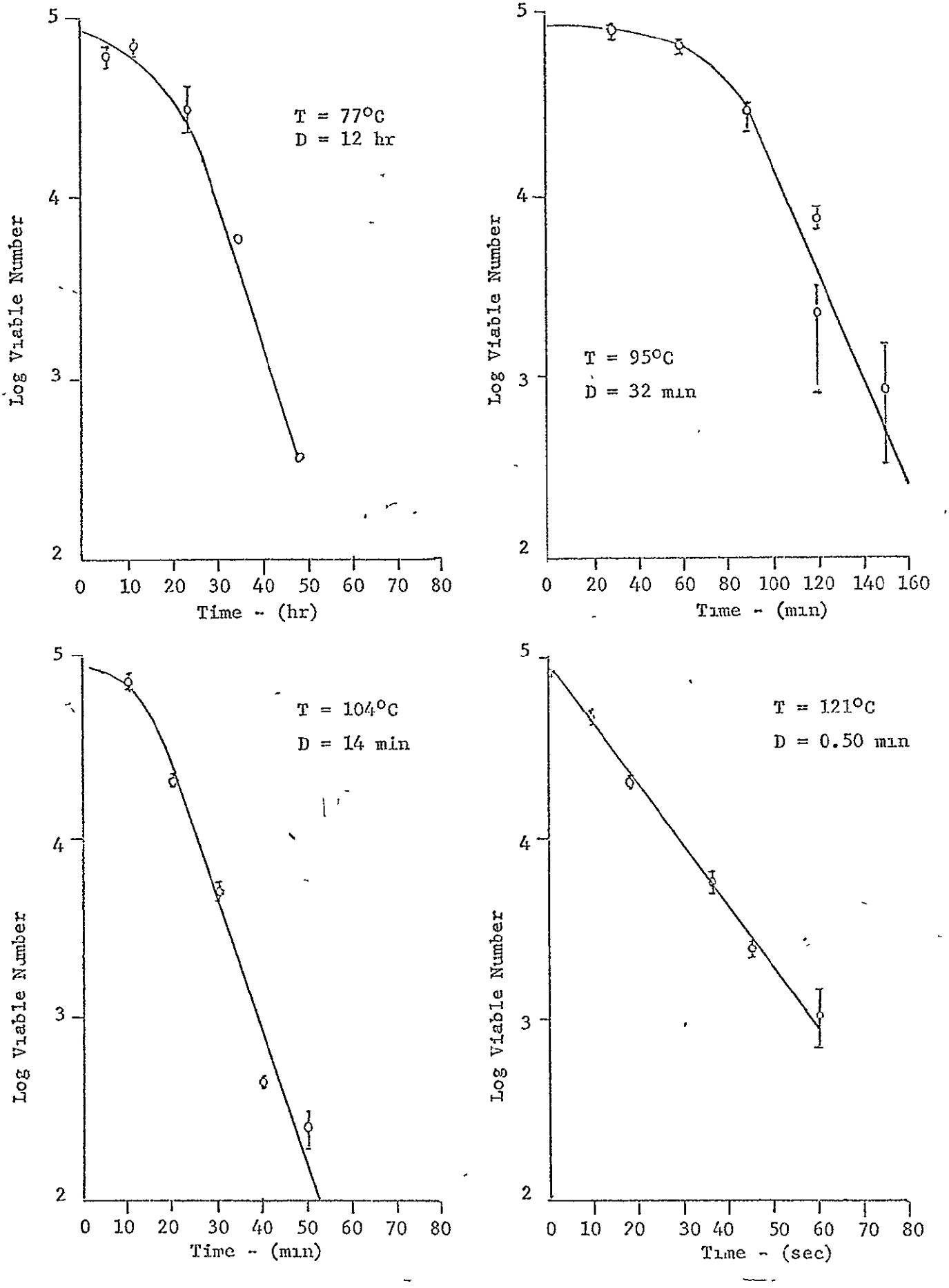

Figure 5. Wet Sterilization Curves (Data from Fox, Eder and Pflug 1967)

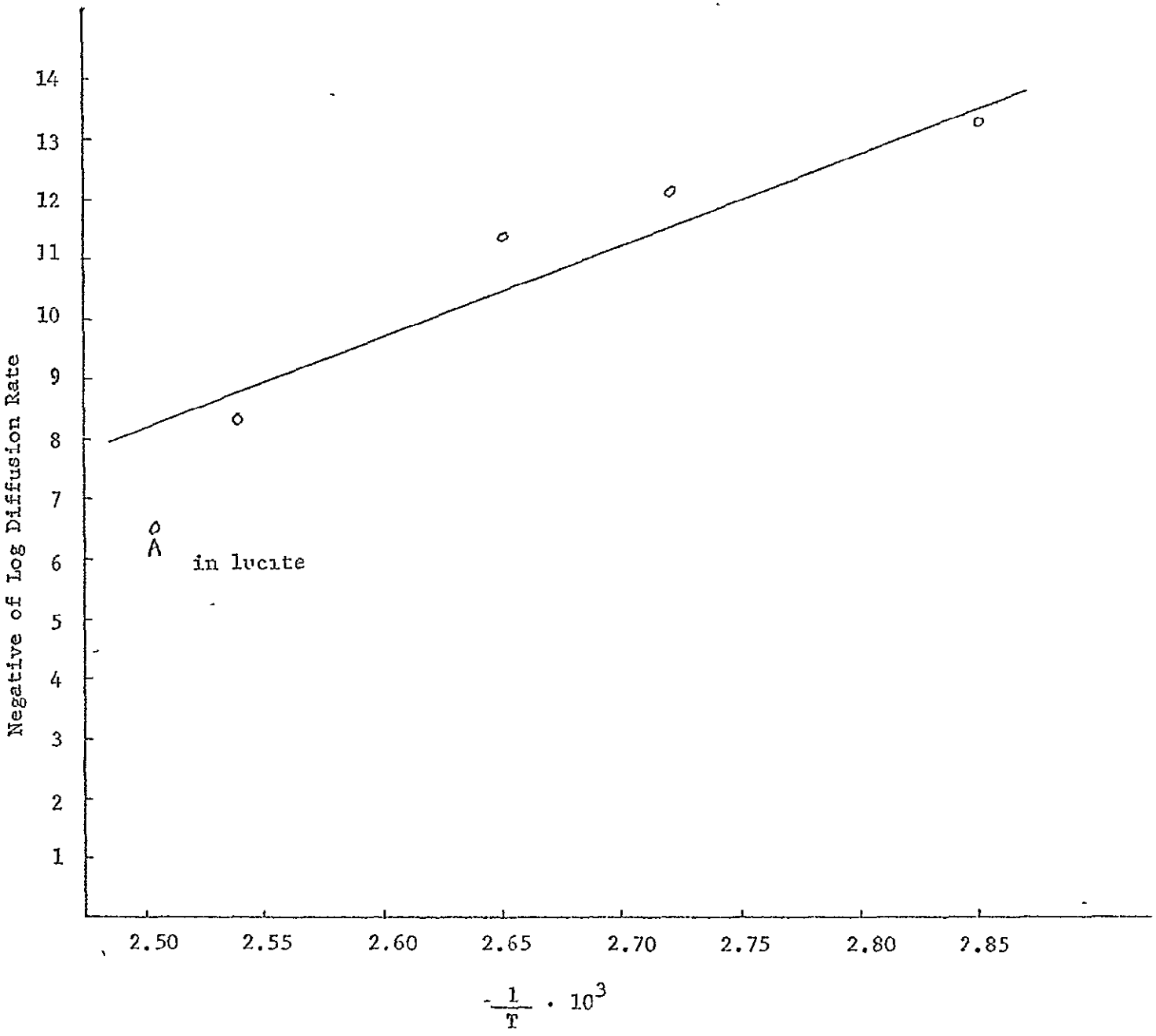

Figure 6. |log D| plotted versus 1/T
(Estimates based on wet heat data by Fox, Eder, Pflug)
(A estimate from dry heat, by Angelotti)

IMPLICATIONS OF THE MODEL

The correlation of water activity with spore resistance indicates that the death mechanism is a denaturation of some vital protein. Much the same correlation has been observed between protein denaturation and water content. Furthermore, the reaction is generally of first-order kinetics, resulting in a logarithmic curve such as is frequently seen for thermal die-off of spore populations.

The vital protein is clearly in the cytoplasm, since the outer portions of the spore, shedded during germination, are not vital. This outer portion, the cortex, appears to squeeze water from the cytoplasm during the formation of the spore, and thereby provides that there will be a residual concentration that is near the optimum for heat resistance.

Part of the heat resistance of spores has been shown to be due to calcium dipicolinate, manufactured during the formation of the spore, and present in the cytoplasm. Ca-DPA is a chelating agent. Presumably, the vital protein has sensitive bonds that are protected by Ca-DPA and other sensitive bonds protected by water molecules.

Germination of the spore presumably requires that the protein be rid of these protections. Excess water can remove the Ca-DPA, the attached water molecules may separate thermally (heat shock). These separations, if reversible, would be of little help to germination unless the protecting molecules stripped from the protein were to leave the cytoplasm. This is the argument for diffusion: it allows proteins to react with enzymes, etc., in the germination process without hindrance from the former bond-protecting agents. At the same time, the proteins become more sensitive to heat.

Diffusion is the random movement of molecules. It is characterized by straight-line paths between molecule interactions, and arbitrary change of direction after the interaction. The net result of the random motion is a movement of molecules from regions of high density to regions of low density. Frequently, these interactions are mere collisions. In such a case, the diffusion constant D is proportional to

temperature. A less frequent situation is where the collisions involve chemical reactions. The molecule moves in a straight line, collides and "sticks" to a fixed obstacle, is freed by the action of heat, and moves off in an arbitrary direction. This kind of diffusion, characterized by a diffusion constant as given in Eq. 5, appears to fit the process of water diffusing through the spore cortex.

The environmental conditions prevailing during spore formation have been shown to affect the subsequent resistance of the spore to heat. This, too, is as the model would imply. There is no known mechanism by which the spore can control the water concentration in the cortex and spore coats. As a result, the moisture content of these regions will vary so that they are in equilibrium with external conditions. When the spore is heated, the contents of these regions can diffuse inward and outward to affect the heat sensitivity of the spore.

These two mechanisms - diffusion and chemical reactions of proteins - appear responsible for the survival probability of spores as a function of humidity, temperature and time. Much work remains in the analysis of their quantitative aspects. What are the equilibrium moisture contents of spores? What is the water distribution inside the spore? What are the surface transfer properties? Does the Ca-DPA diffuse, too? What is the protein denaturation reaction that occurs? How many protein molecules must denature before the spore becomes nonviable? The answers to these questions require further study. Many previous experiments, unfortunately, are of little help since not all the pertinent variables were measured.

RECOMMENDATIONS FOR FUTURE WORK

1. Moisture Content Analysis

Measurements of the moisture content of spores are needed. For ease in comparing results of different environments, only one species (B. subtilis var. niger is the accepted norm) should be used and standard harvesting, washing, and drying procedures employed. Selection of procedures is a subject for discussion, but standardization will permit studies of the results to be concentrated on the environmental effects.

The environments to which the spores are equilibrated can vary in temperature, in humidity, and in length of time of equilibration. Down-side and up-side equilibration need separate studies, in view of the "hysteresis" effect observed. The rate at which spores give off water during an analysis, together with the cumulative water emission, should be measured.

These measurements should be analyzed to see whether they conform to the hypotheses discussed in this report. Evaluations of diffusion coefficients and surface transfer effects should be possible.

2. Correlation of Moisture and Sterilizability

Dry heat sterilization, with moisture content as a parameter, has been measured with uncertain results. The understanding gained from the analysis above should provide benchmarks for future measurements of spore sterilization rates. The early work sometimes suffered from a lack of determination of the pressure or the relative humidity during sterilization. The substrate deserves attention: if it has good water transference properties it can affect the experiment significantly.

With knowledge of moisture transfer properties in the spore, on its surface, and through the environment, it should be possible to

design crucial experiments where the moisture content and temperature of the spores are known quantities. The die-off of spores under these conditions should be measured. Such experiments include:

(a) Spores in vacuum. The moisture transfer outside the spores is a relatively easy calculation.

(b) Spores in non-permeable materials (e.g. epoxy). With different initial moisture contents, spores hould show different die-offs, but each experiment should provide nearly logarithmic curves.

(c) Spores in air. Moisture transfer is affected by the relative humidity and this can be varied in a sequence of experiments.

(d) Spores of different initial A_w in air.

REFERENCES

1. Alderton, G.; Thompson, P. A.; and Snell, N.: "Heat Adaptation and Ion Exchange in Bacillus Megaterium Spores"(1964) Science 143, 141-143.

2. Angelotti, R.: "Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components" Fourth Quarterly Report of Progress on Research Project R-36-015-001 March 1966, 1967, 1968.

3. Black, S. H.; Hashimoto, T.; and Gerhardt, P.· "Calcium Reversal of the Heat Susceptibility and Dipicolinate Deficiency of Spores Formed Endotrophically in Water" (1960) Can. J. Microbiol 6 212-224.

4. Clark, W. M.· "Topics in Physical Chemistry" p. 138 (1948) The Williams and Wilkins Co., Baltimore.

5. Halvorson, H. and Howitt, C.· "The Role of DPA in Bacterial Spores" (1961) Spores II H. Halvorson (ed) Burges Publishing Co., Minneapolis.

6. Hunnell, J. W. and Ordall, Z. J.: "Cytological and Chemical Changes in Heat Killed and Germinated Bacterial Spores" (1961) Spores II H. Halvorson,(ed) Burges Publishing Co., Minneapolis.

7. Lewis, J. C.; Snell, N. S.; and Burr, H. K.: "Water Permeability of Bacterial Spores and the Concept of a Contractile Cortex" (1960) Science 132· 544-545.

8. Maynall, B. H. and Robinow, C. F.· "Observations with the Electron Microscope on the Organization of the Cortex of Resting and Germinating Spores of Bacillus Megaterium" (1957) J. Appl.Bacteriol. 20 333-341.

9. Murrell, W. G. and Scott, W. J.· "The Heat Resistance of Bacterial Spores at Various Water Activities" J. Gen. Microbiol. (1966) 43, 411-425.

10. Slepecky, R. and Foster, J. W.· "Alteration in Metal Content of Spore of Bacillus Megaterium and the Effect on Some Spore Properties" (1959) J. Bacteriol.78 117-123.

11. Vinter, V.· "The Formation of Cystine-Rich Structure in Sporulating Cells and its Possible Role in the Resistance of Spores" (1961) Spores II H. Halvorson (ed) Burges Publishing Co., Minneapolis.

12. Warth, A. D.; Ohye, D. F.; and Murrell, W. G.· "The Composition and Structure of Bacterial Spores" (1963) J. Cell. Bio. 16 579-592.

13. Young, I. E.· "A Relationship Between the Free Amino Acid Pool, Dipicolinic Acid and Calcium from Resting Spores of Bacillus megaterium" (1959) Can. J. Microbiol. 5 197-202.

APPENDIX C

An Analytical Basis for Assaying Buried Biological Contamination

AN ANALYTICAL BASIS FOR ASSAYING BURIED BIOLOGICAL CONTAMINATION

Interim Report

Contract NASw-1734

for

National Aeronautics and Space Administration

Prepared by

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January 1969

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SECTION 1 - Introduction

SUMMARY AND EVALUATION

This document prescribes and evaluates a procedure for estimating upper bounds on the mean concentration of viable organisms buried within individual spacecraft materials.*

Presented herein is an analysis of a procedure for assaying biological contamination buried or embedded in spacecraft materials. The procedure requires the controlled fracture of representative samples of a material whose buried loading is of interest. Each sample is tested for biological contamination on the totality of surfaces exposed as a result of the fracturing process. The basic datum or observation consists of the proportion of samples which yield contamination upon culturing. Conventional statistical techniques, combined with an assumed relation between the mean concentration of organisms buried within the material and the observed datum, produce an upper bound estimate for the unknown mean concentration, expressed to any prescribed level of confidence. In principle, the "conservativeness" of the resulting estimate is directly related to the sample size and the amount of surface area exposed by fracture; as the sample size and/or exposed area increase(s) the difference between the estimate and the unknown mean load tends to decrease.

The procedure, if feasible in terms of accuracies derived, engineering practicality and economics, would be very useful in the specification of realistic spacecraft sterilization requirements. This follows from the fact that sterilization requirements are quite sensitive to the release of buried contamination. Significant decreases in these requirements may be possible if realistic estimates of the buried bio-loads are made available. Conventional bio-assay techniques are impractical for most applications to spacecraft materials since they require that the materials be either pulverized or dissolved. The procedure discussed herein requires neither of these actions and, moreover, requires no direct counting of viable organisms.

There are potential shortcomings in the proposed procedure. In particular, there may be practical engineering difficulties or cost considerations which limit the application of the technique. Moreover, the accuracies resulting from its application could turn out to be less than desired. For these reasons, it is important that tests and further analyses be conducted to resolve these questions before steps are taken to operationally implement the procedure.

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*The concept underlying the procedure discussed herein was originally suggested by L. Hall, Office of Biosciences, National Aeronautics and Space Administration.

SECTION 2 - Background

SENSITIVITY OF MISSION REQUIREMENTS TO THE RELEASE OF BURIED CONTAMINATION

Sterilization requirements for individual lander missions are quite sensitive to the release characteristics of buried contamination. Effective techniques for assaying buried contamination could lead to substantial decreases in these requirements.

Recent analyses indicate that spacecraft sterilization requirements for planetary lander missions are quite sensitive to the release characteristics of contamination buried (embedded) within spacecraft materials.[1] In fact, under the majority of presumably realistic situations studied, the threat of buried contamination was the controlling factor in the determination of sterilization requirements. Within the context of the subject analyses, this result was attributed to the relatively high resistance of buried contamination to sterilizing temperatures, as compared with resistances of contamination located on open and between mated surfaces (resistance being represented by the D-value parameter of the exponential survival curve).

The relatively high resistance of buried contamination presently assumed (viz. $D_{125°C}$ = 5 hours) was not the only factor contributing to the dominance of buried contamination in the determination of sterilization requirements. Two other major contributors were (1) the break-up characteristics of spacecraft materials containing buried contamination and (2) the amount of contamination actually buried within these materials, i.e. the threat of buried contamination is directly related to the existing amount of contamination and its accessibility to a planetary environment upon impact. The lack of definitive data relating to these factors presently necessitates a pessimistic view of their quantitative effects on sterilization requirements. Therefore, in exploring alternatives for decreasing sterilization requirements on individual lander missions, consideration should be given to justifying less pessimistic representations of the effects of these factors. This could be accomplished by determining more realistic estimates of spacecraft break-up characteristics and buried bio-loadings.

The break-up characteristics of spacecraft materials should be and are being investigated.[2] However, there are inherent difficulties associated with the quantification of this aspect of the buried biological threat. For example, there are practical problems associated with measuring the amount of break-up and relating it to flight path parameters such as impact velocity. In fact, very little is presently known about the fracturing characteristics of the many varieties of spacecraft materials. In many respects, determination of the amount of buried contamination is less complicated than the break-up problem. For example, the magnitude of the buried bio-load has nothing to do with the uncertainties of the mission flight path whereas the amount of spacecraft break-up is intimately related to the mission flight path parameters. The extensive background material in existence concerning biological loadings and their measurement, also suggests it to be a more fertile area of investigation.

SECTION 2 - Background

DRAWBACKS OF CONVENTIONAL BIO-ASSAY TECHNIQUES

Conventional techniques for assaying buried contamination are impractical for applications to most spacecraft materials since they require that materials be either pulverized or dissolved.

Standard laboratory procedures for the detection and enumeration of buried contamination fall into two major categories. One class of procedures requires that the subject material be broken into very small pieces (i.e. pulverized) and that direct counts of the exposed organisms be made. A second class of procedures requires that the material under investigation be dissolved in a suitable solvent which is non-toxic to the buried organisms. This second procedure also involves the subsequent counting of exposed organisms.

Several drawbacks are inherent in the application of the above techniques, especially where spacecraft materials are involved. Basically, there are serious questions relating to the practicality of pulverizing or dissolving most spacecraft materials (as opposed to laboratory application of those techniques which mainly involve materials that can be appropriately pulverized or dissolved). In the particular case of pulverization techniques, the basic objective is to reduce the solid to particles of a size which essentially releases all organisms that are present without damaging the individual cells. Significant numbers of organisms usually go undetected since the chance of releasing all of them is very small. In addition, it has been found that the pulverization process itself damages or "kills" significant numbers of organisms, thus rendering them undetectable. For the most part, these two engineering problems prohibit a precise assay; if the pulverization is complete enough then it is likely that a significant number of organisms will be damaged in the process. The analogous problem associated with the use of solvents is that very few types of materials can be dissolved without using combinations of heat, pressure and chemicals which destroy the buried organisms. Finally, both classes of procedures are appropriate only when counting high concentrations of contamination. They are ineffective or, at best, inefficient when applied to the low numbers of organisms buried within aerospace hardware. Their reliability for measuring low densities, (e.g. less than one organism per cubic centimeter) has not been adequately established.

SECTION 3 - Development of the Bio-Assay Procedure

BASIC CONCEPTS AND ASSUMPTIONS UNDERLYING THE PROPOSED PROCEDURE

The proposed procedure is premised upon a more-or-less uniform distribution of buried contamination and the release of the biological contents of a subvolume of material exposed through fracturing.

Suppose a given volume of homogeneous spacecraft material which contains buried contamination is fractured into several distinct pieces. It is reasonable to assume that the fracturing process effectively exposes the biological contents of a subvolume, V_e, of the interior of the material. Analysis of recent fracturing experiments[1] indicates that Expression (1) on the facing page is an acceptable representation of the exposed subvolume. In this expression A_e denotes the surface area newly exposed as a result of fracturing and λ denotes an effective depth of penetration, i.e., the depth beneath the exposed surface to which previously buried contamination is released. For convenience, λ is designated the "exposure depth coefficient". This concept of a subvolume exposed by fracturing was applied to the data obtained from the previously mentioned experiments. Estimates of λ evolved which ranged between one (1) and three (3) microns. This range has intiuitive appeal in that it encompasses the mean diameter of microbial spores.

It is reasonable to assume that contamination buried within an homogeneous material is, for the most part, uniformly distributed throughout the interior. Moreover, assuming a standardization or uniformity of parts production procedures suggests that the concentration of contamination per unit volume of material is randomly distributed about some fixed value, C. These observations, along with recognition of an essentially unending source of contamination in production environments suggest the Poisson distribution as an appropriate formulation for describing the dispersion of buried contamination within homogeneous solid materials. This being the case, the probability that exactly K organisms are contained within the exposed subvolume V_e is given by Expression (2). In this expression N_e denotes the number of organisms exposed through the fracturing process.

The preceding two assumptions on the effective subvolume of material exposed by fracturing and the uniform dispersion of biological contamination within the selected materials constitute the basis for the analytical bioassay procedure presented and applied in the remainder of this report.

Effective Subvolume of Material Exposed Through Fracturing

$$V_e = \lambda A_e \tag{1}$$

λ - Exposure depth coefficient
A_e - Exposed surface area

Probability that Exactly K Organisms are Exposed Through Fracturing

$$P\left\{N_e = K\right\} = \frac{(\lambda A_e C)^K}{K!} e^{-\lambda A_e C} \tag{2}$$

$$K = 0, 1, 2 \cdots$$

C = Mean concentration of organisms per unit volume of material.

SECTION 3 - Development of the Bio-assay Procedure

THE BASIC MATHEMATICAL RELATIONSHIP (MODEL)

The probability that buried contamination will be exposed through fracturing is obtained from the assumed Poisson distribution. The resulting representation is the basic relationship or model underlying the proposed bio-assay procedure.

The probability that buried contamination will be exposed when a solid material is fractured can be obtained from the previously developed form of the Poisson distribution (Expression (2)). It coincides with the Poisson probability, p, that at least one viable organism is contained in the effective subvolume of material exposed as a result of the fracturing process. The expression for this probability, indicated by Expression (3) on the facing page, constitutes the basic relationship underlying the proposed bio-assay procedure. For convenience, it is rewritten in Expression (4) as a relationship which specifies the mean concentration, C, of organisms per unit volume of material in terms of the parameters λ , A_e, and p.

In principle, if values of λ , A_e, and p are specified, then the unknown concentration, C, can be determined on the basis of Expression (4). In practice, none of the above parameters can be determined or controlled exactly. The exposure depth coefficient, λ , can be estimated on the basis of experimental laboratory data on varied materials. (The previously mentioned estimates of λ were determined on the basis of Expression (3), controlled values of C and A_e and experimentally obtained estimates of p.) Assuming that the area, A_e, exposed through fracturing can be controlled sufficiently, estimates of p can be obtained experimentally. These estimates of p can be converted to upper bound estimates of C to any level of confidence on the basis of Expression (4) and standard statistical estimation techniques.

Probability that Buried Contamination is Exposed Through Fracturing

$$
\begin{aligned}
p &= P\left\{ N_e \geq 1 \right\} \\
&= 1 - P\left\{ N_e = 0 \right\} \\
&= 1 - e^{-\lambda A_e C}
\end{aligned}
\tag{3}
$$

The Mean Concentration of Buried Contamination Per Unit Volume of Material

$$
C = \frac{-\ell n\,(1 - p)}{\lambda A_e} \tag{4}
$$

SECTION 3 - Development of the Bio-assay Procedure

STATISTICAL LOAD ESTIMATION PROCESS

An upper bound estimate of the mean bio-load, expressible to any level of confidence, is determined from Expression (5) and experimentally obtained estimates of the probability that buried contamination will be exposed by fracturing.

Suppose that s samples of a specific spacecraft material are selected at random (e.g., the samples could be piece parts constructed from a particular homogeneous material). Further, suppose that each sample is fractured exposing a predetermined amount of new surface area, the same area being generated for all samples. Each sample is classified as "positive" if and only if biological contamination is found on the newly exposed surface area upon culturing. Assuming all samples are processed according to a fixed experimental procedure, the number of positives is Binomially distributed with "success" parameter p, as defined in Expression (3). Hence, the proportion, $\hat{p}$, of positives has an average or mean value p and is distributed as specified in Expression (5) on the facing page. Knowledge of the distribution of $\hat{p}$ allows for the determination of arbitrary confidence intervals for the unknown p, expressed in terms of observed values of $\hat{p}$. Appendix A contains a procedure for obtaining confidence intervals for p in terms of $\hat{p}$ and the sample size s. Figures I - B through IV - B of Appendix B are displays of 50, 80, 90 and 95 percent confidence intervals for p, respectively, determined using the procedures outlined in Appendix A. The various curves in each figure correspond to selected sample sizes.

Since the mean bio-concentration, C, specified in Expression (4) is an increasing function of p, upper confidence limits for p can be transformed, via this expression into corresponding confidence limits on C. Since this transformation involves the exposed area as well the sample size, a given confidence limit on p transforms into a distinct confidence limit on C for each value of exposed area. Figure V - B through VIII - B of Appendix B display the resulting upper confidence limits on C, corresponding to selected values of s, A_e and the observed proportion, $\hat{p}$, of positives. It should be noted that the lower confidence limits on p are not transformed into limits on C in these figures since primary concern here is with upper bound limits.

Figures V - B through VIII - B of Appendix B provide the necessary tools for testing and implementing the proposed analytical bio-assay procedure. The remainder of this report contains a step-by-step procedure for applying these figures, an illustrative application and a discussion of potential sources of load estimation error associated with the procedure.

$$p\left\{\hat{p} = \frac{k}{s}\right\} = \binom{s}{k} p^{k}(1-p)^{s-k} \qquad (5)$$

$k = 0, 1, 2, \text{---}, s$

SECTION 4. - Applications of the Bio-assay Procedure

SUMMARY PROCEDURE AND ILLUSTRATION

The preceding development is converted to an operational procedure using Figures V-B thru VIII-B of Appendix B.

On the basis of the preceding development, a protocol for establishing upper bound estimates of the mean bio-load buried within a given spacecraft material is summarized as follows

1. Determine an appropriate combination of sample size, s, and area to be exposed, A_e (this decision is based, for the most part, upon "fracturability" and cost considerations)

2. Select at random a number, s, of samples of the given spacecraft material.

3. Fracture each sample so as to yield the selected amount, A_e, of newly exposed area.

4. For each sample, establish whether viable organisms were exposed upon fracture. Let $\hat{p}$ denote the proportion of samples which yield contamination, i.e. $\hat{p}$ is the proportion of positive samples.

5. On the basis of s, A_e and $\hat{p}$ and the desired level of confidence read the corresponding upper bound estimate of the mean bio-load on the appropriate graph from Figures V-B through VIII-B of Appendix B.

The indicated graphs do not allow for arbitrary selections of the sample size and exposed area. In the event that curves corresponding to other values of these parameters are needed, they can be determined from Expression (4) and Figures I-B through IV-B of Appendix B.

To illustrate the procedure, suppose that 25 samples of a given spacecraft material are selected and each sample is fractured, yielding an exposed area of 2.5 square inches. Suppose that 15 of these samples display contamination on the newly exposed surfaces. Finally, assume that an upper bound estimate of the mean bio-concentration is desired at the 90% confidence level. It is determined from Figure VIII-B of Appendix B, i.e. the graphical display corresponding to the 90% confidence level. The appropriate curve in this figure is the one corresponding to the given sample size, i.e. s=25. The observed proportion of positive samples is given by $\hat{p} = 15/25 = 0.6$. This value on the horizontal axis of the figure determines the appropriate point on the s=25 curve. Using the vertical scale corresponding to the

exposed area $A_e = 2.5$ in^2, an upper bound estimate of the mean bio-concentration is seen to be less than 1.04×10^4 viable organisms per cubic inch with 90% confidence. This concentration, when multiplied by the total volume of subject material on the spacecraft, produces an upper bound estimate of the bio-load buried within the given material.

SECTION 5 - A Preliminary Evaluation of the Bio-assay Procedure

SENSITIVITY OF LOAD ESTIMATES TO THE CONTROL AND MEASUREMENT OF λ AND A_e

To a first order approximation, percent errors in the measurement of either λ or A_e induce equivalent percent errors in estimates of the mean concentration of buried contamination.

As indicated in Expression (4), the mean concentration of buried contamination is inversely proportional both to the exposure depth coefficient, λ, and to the exposed area, A_e. Therefore, to a first order approximation, percent errors in the measurement of either one of these parameters result in identical percent errors in the mean concentration of buried contamination. Although this statement cannot be extended beyond certain limits, it does provide an approximate quantitative measure of the effects of measurement errors in λ and A_e on estimates of the unknown mean concentration, C.

As noted earlier, experimentally determined estimates of λ ranged between one and three microns for a specific material (lucite) and particular measurement procedures. If the "true" value of λ lies within this range for all spacecraft materials then a maximum of 300% error in λ is possible (i.e., assuming λ equals 3 microns but is estimated to be 1 micron). Assuming that order or magnitude estimates of spacecraft bio-loads are sufficient for most applications, errors of the above magnitude appear to be acceptable. In any case, taking λ as the lower limit of the range of estimates (i.e., 1 micron) provides more concervative upper bound estimates of C than would any other value selected in the given range; this is consistent with sterility assurance. There is no question, however, that estimates of λ corresponding to materials other than lucite is desirable, if not mandatory.

There is a sparsity of both theoretical and empirical data on the control and measurement of surface areas exposed by fracturing materials of the types used in spacecraft construction. Moreover, the implications of a given distribution of measurement errors in A_e are, at best, difficult to derive on a statistical basis owing to the relatively complex relationship between A_e, the observed datum, $\hat{p}$, and the estimated mean biological concentration. For these reasons, it is difficult to speculate on the errors introduced into bio-assay estimates as a result of incorrect measurements of exposed areas. The previously referenced Pheonix experiments [1] failed to provide sufficient data for resolving these questions completely, even as applied to lucite. However, evaluation of the experimental procedures and the resulting data does suggest that, for this particular material, the area control and measurement procedures used along with selecting λ equal to one micron is adequate for present purposes. Here again, extrapolation to other materials may not be valid; hence, additional data in this regard is warranted.

SECTION 5 - A Preliminary Evaluation of the Bio-Assay Procedure

ADDITIONAL SOURCES OF POTENTIAL ERROR

Potential sources of error related to deficiencies in the sampling and culturing processes as well as the analytical model itself indicate the need for controlled tests of the bio-assay procedure.

The proposed procedure evolved, in part, from the assumption of a Poisson distribution of viable organisms within the interiors of materials being assayed. Although this representation has intuitive appeal for most applications, a test and validation phase is nevertheless necessary before implementation is considered.

Estimation errors are likely to occur if improper sampling, and/or culturing procedures are followed. In sampling material, care must be taken to insure that a representative cross-section is selected, i.e., samples independently taken from distinct batches of the given material. Otherwise, the selected sample size could be insufficient for attaining a desired confidence level. The culturing procedure is intimately connected with the exposure depth coefficient, λ , since the depth of penetration is likely to vary with the nature or type of culture medium. For any given depth of penetration, however, the culturing process should be capable of detecting all viable organisms which are exposed.

It is important to note that the proposed bio-assay procedure, if successful, produces upper bound estimates of the mean concentration taken over the total population of the sample material under investigation. This is less than desirable from the standpoint of application to sterilization requirements for individual lander missions. For example, it is possible, though quite unlikely, that the dispersion of concentration from sample to sample is very large. If so, an upper bound estimate of the mean concentration to any level of confidence could have a relatively high probability of being less than the concentration of a randomly selected sample and spacecraft. Further testing and analysis are warranted on this basis alone.

SECTION 6 - Recommendations

THE NEED FOR TESTING AND FURTHER ANALYSIS

Tests and additional analysis of the usefulness, engineering practicality and economics of the proposed procedure are recommended prior to implementation.

Application of the proposed bio-assay procedure to any given spacecraft material yields an upper bound estimate, $\bar{C}$, of an unknown mean concentration of buried organisms within the material. The usefulness of this estimate depends both upon its accuracy and the amount of information it contains. Acceptable quantitative measures of the effects of the previously indicated error sources on the accuracy of $\bar{C}$ must be determined. Further, the procedure must be shown to yield information which is needed and presently unavailable (e.g., estimates which are consistently greater than already known upper bounds are of little or no use). Attainment of these objectives requires the accomplishment of appropriate tests and analyses. For example, experimental test applications should be conducted on various classes of materials wherein the buried loadings are controlled (known). In addition, further analytical studies should be pursued which relate to the effects of (1) errors in the measurement of A_e and λ, (2) the assumption of uniformly distributed organisms and (3) the errors introduced by virtue of the fact that estimates relate to the mean concentration rather than the particular concentration going aloft in a spacecraft.

A research area requiring investigation is the engineering practicality of applying the proposed procedure. It must be determined, for example, whether appropriate control and measurement of the area exposed, A_e, is feasible. (The requirements on this accuracy should evolve from the efforts discussed in the preceding paragraph.) The practicality of appropriately culturing the exposed surface areas also requires additional study.

Finally, consideration must be given to the economics of the proposed procedure. For the most part, this should be based upon the cost of securing and processing sufficient numbers and varieties of sample spacecraft materials.

APPENDIX A

Computation of Confidence Intervals for Binomial "Success" Probability

APPENDIX A - Computation of Confidence Intervals for a Binomial "Success" Probability

Confidence intervals can be established for the "success" parameter of a Binomial distribution by using an approximating normal distribution. These intervals can then be transformed into upper and lower bounds upon the bio-load.

As indicated in the main body of this report, the confidence limits established for the mean bio-load are directly related to the confidence intervals for a Binomially distributed variable $\hat{p}$, which represents the observed proportions of times contamination was detected in the load estimation procedure. Expression (A1) on the facing page expresses the definition of a confidence interval. The interpretation of this expression is; for a confidence of $1-\alpha$, the probability that the parameter p is contained within the interval (p_L, p_u) is greater than or equal to $1-\alpha$. For the particular use of a Binomial distribution, Expression (A1) is solved by first considering expressions (A2) and (A3). In these expressions, the parameters p_u and p_L take on the largest possible value such that the given inequalities are satisfied. This would then give the $(1-\alpha)$ confidence interval of (p_L, p_u) on the parameter p. However, from a computational point of view, Expression (A2) and (A3) are subject to round-off errors and machine overflow. By using a well-known normal approximation to the Binomial distribution, we can replace Expressions (A2) and (A3) with the corresponding expressions resulting from this approximation. The approximating normal distribution will have mean p and standard deviation

$$\sqrt{\frac{p\ (1-p)}{s}} \quad .$$

Thus p and $\hat{p}$ are related as in Expression (A4) on the facing page. This equation must be solved for p in order to obtain p_L and p_u. It can be shown that Expression (A5) is the result of solving (A4) to obtain p in terms of $\hat{p}$. Expression (A4) with the plus sign corresponds to the upper half of an ellipse which gives the upper confidence limit on p. Likewise, using this expression with the negative sign gives the lower half of this ellipse corresponding to the lower confidence limit on p. A family of these ellipses is shown in Figures (I-B) thru (IV-B) of Appendix B. Since we are interested in upper bounds on the bio-load, Expression (A6) gives the upper bound on the bio-load in terms of p_u.

In the load estimation procedure a value for $\hat{p}$ would be obtained for the particular sample size used. For this value of $\hat{p}$ and for a desired confidence level, p_u would be obtained from Expression (A5).

$$P_r \left\{ p_L \leq p \leq p_u \right\} \geq 1 - \alpha \tag{A1}$$

$$P_r \left\{ \hat{p} > p_u \right\} = \sum_{x=sp_u}^{x=s} \binom{s}{x} p^x (1-p)^{s-x} \leq \frac{\alpha}{2} \tag{A2}$$

$$P_r \left\{ \hat{p} < p_u \right\} = \sum_{x=0}^{x=sp_L} \binom{s}{x} p^x (1-p)^{s-x} \leq \frac{\alpha}{2} \tag{A3}$$

$$\hat{p} = p \pm \delta \sqrt{\frac{p(1-p)}{s}} \tag{A4}$$

$$P_{L,u} = \frac{s}{s + \delta^2} \left\{ \hat{p} + \frac{\delta^2}{2s} \pm \delta \left[\frac{\hat{p}(1-\hat{p})}{s} + \left(\frac{\delta}{2s} \right)^2 \right]^{\frac{1}{2}} \right\} \tag{A5}$$

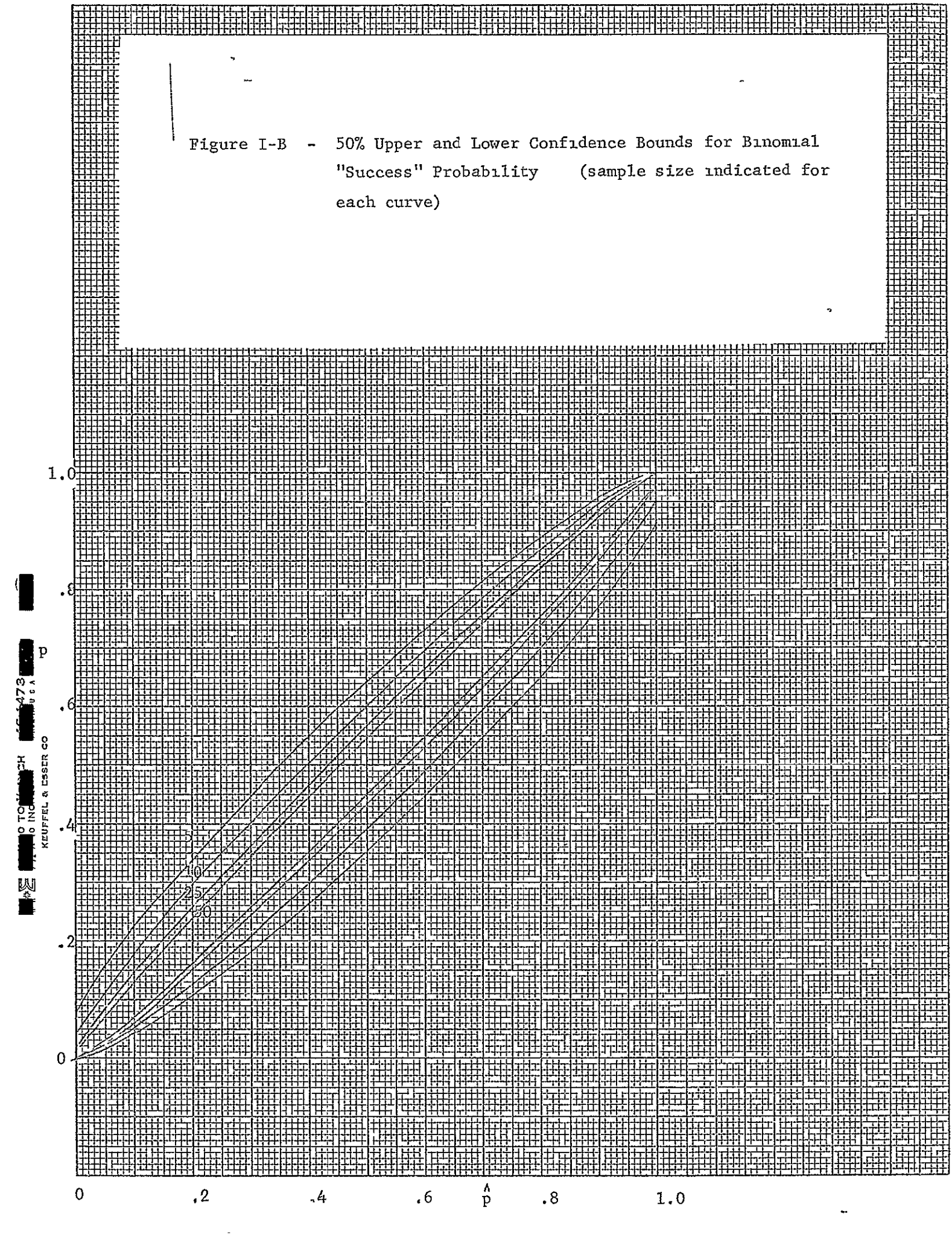
s = sample size

δ = standardized normal deviate

APPENDIX B

Graphical Displays of Confidence Limits

Figure I-B - 50% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)



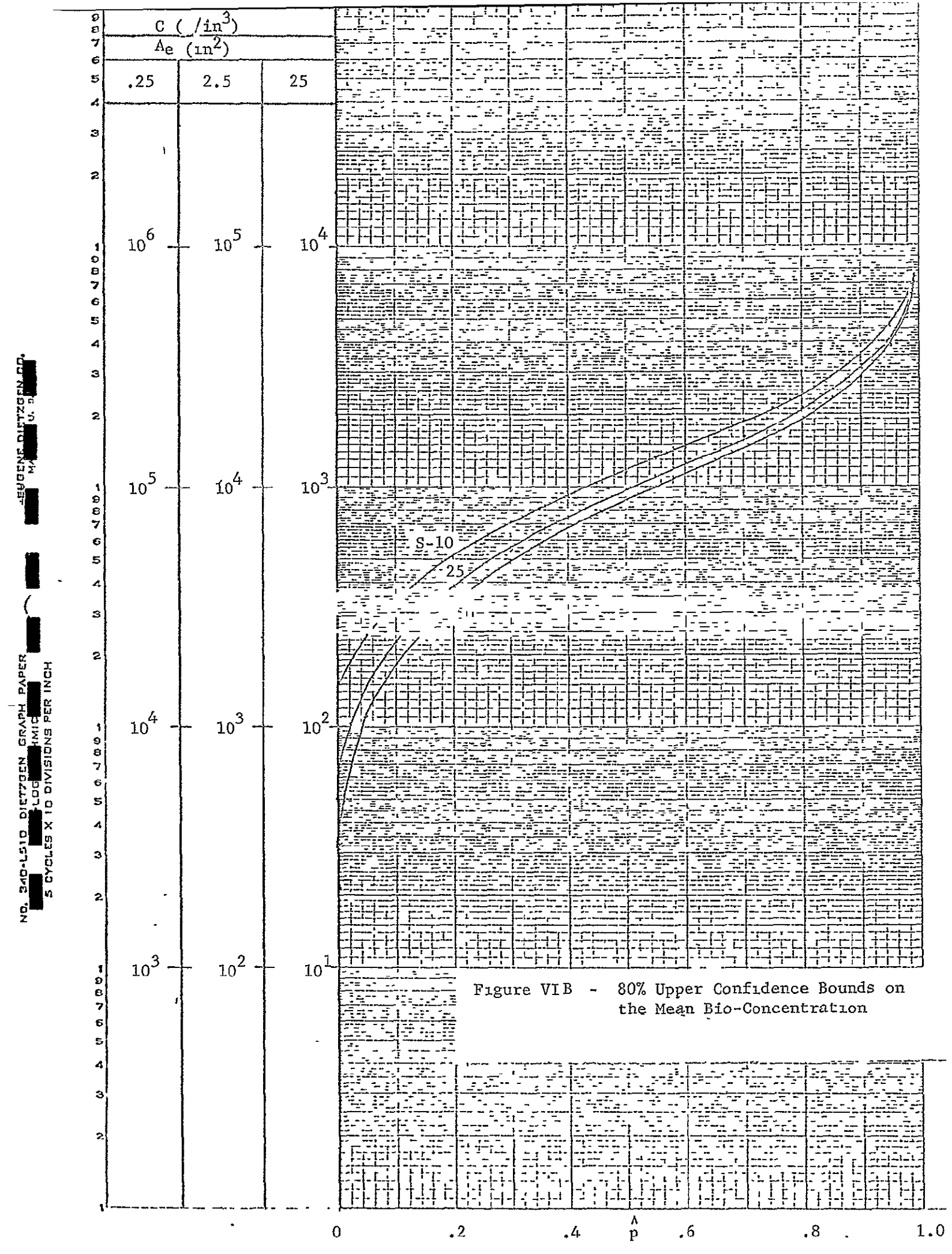


Figure VIB - 80% Upper Confidence Bounds on the Mean Bio-Concentration

Figure II-B - 80% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)

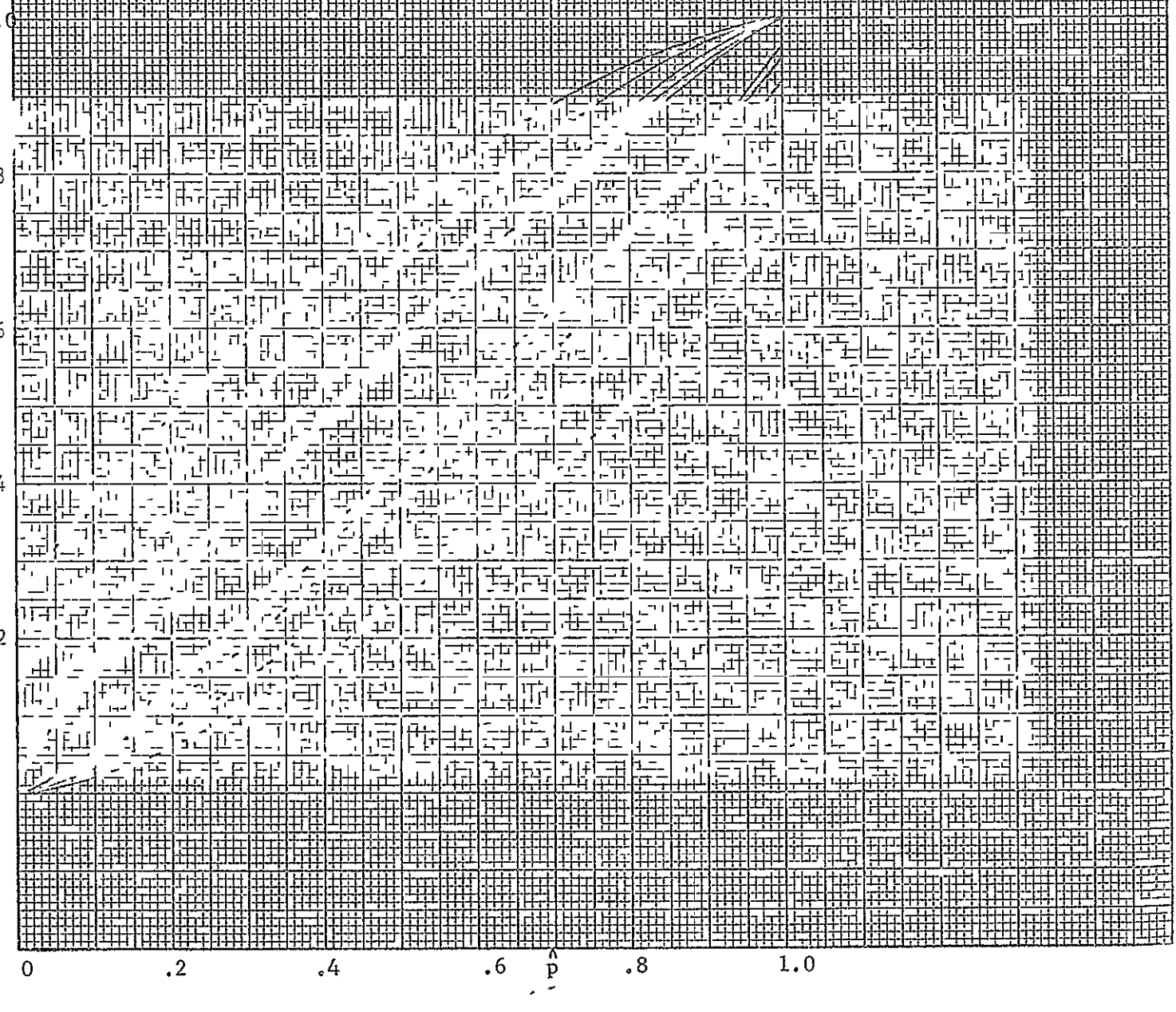


Figure III-B - 90% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)

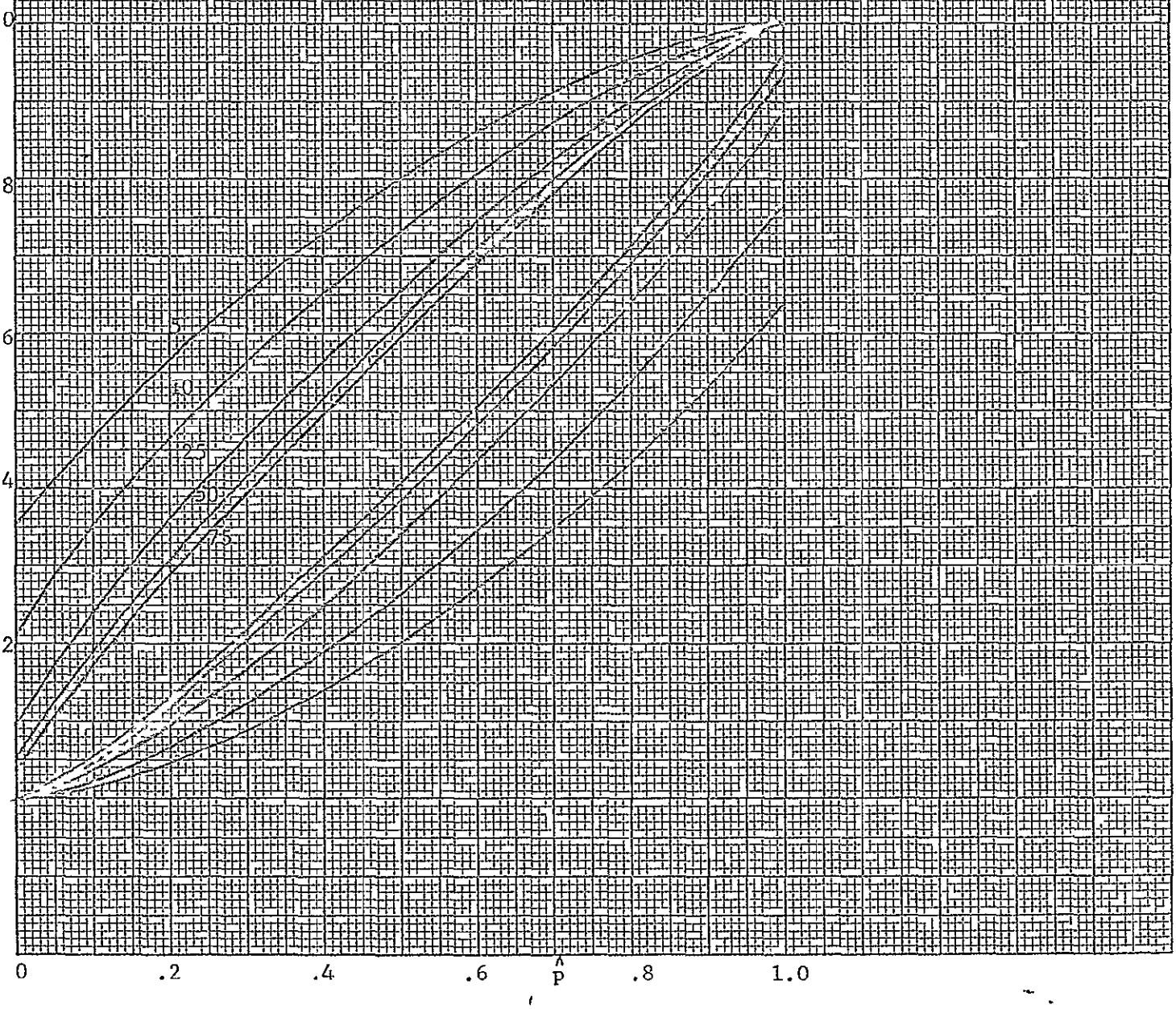
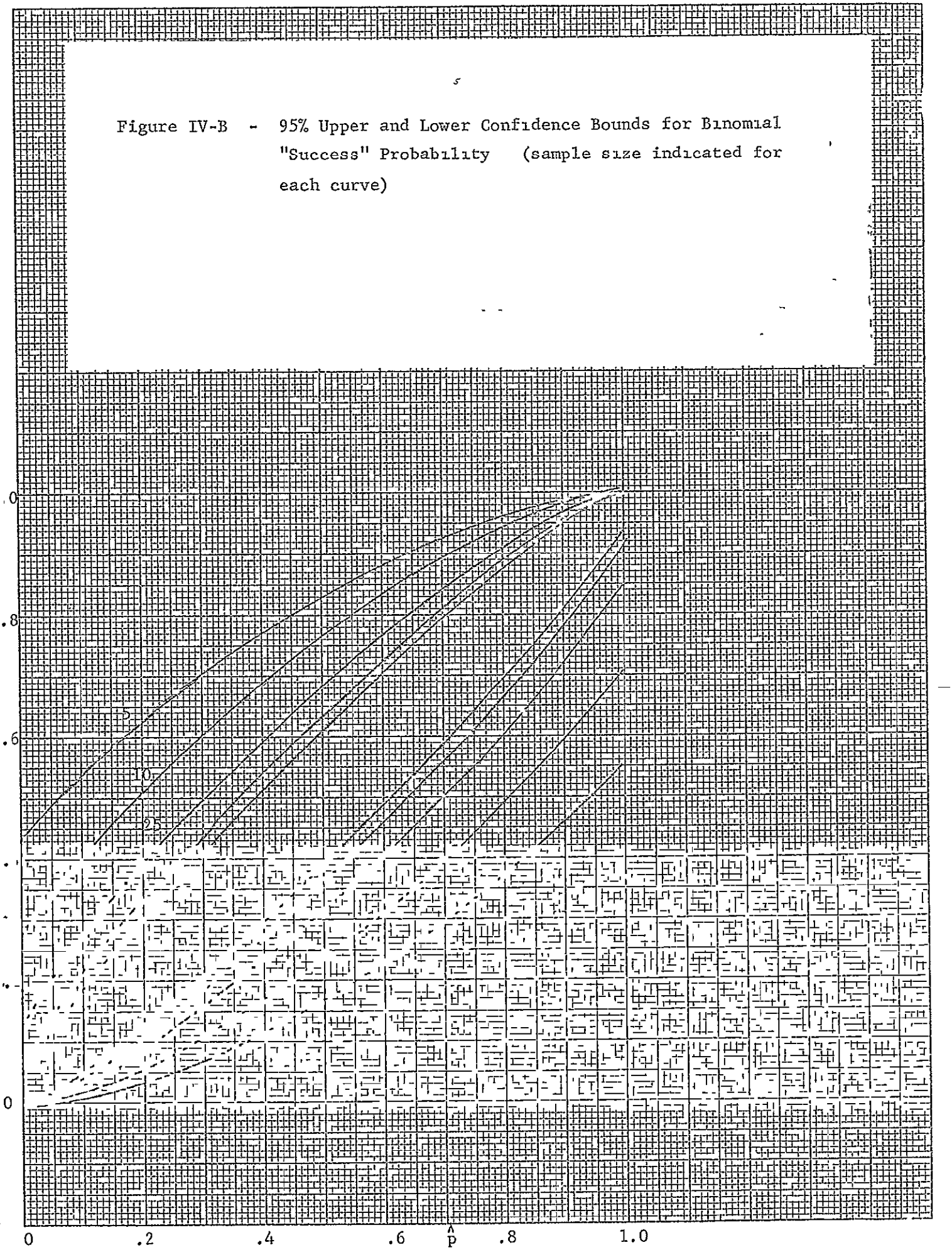


Figure IV-B - 95% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)



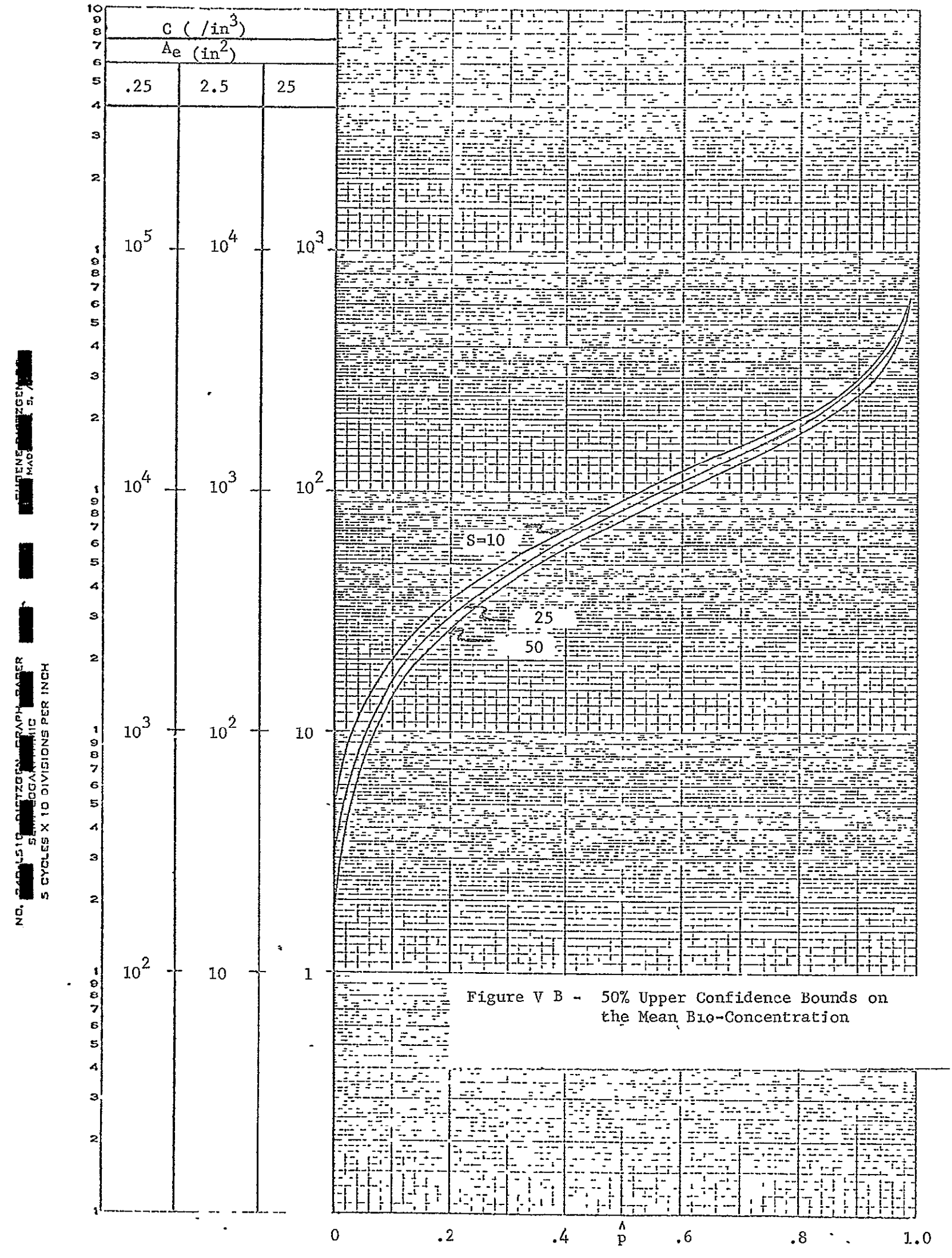


Figure V B - 50% Upper Confidence Bounds on the Mean Bio-Concentration

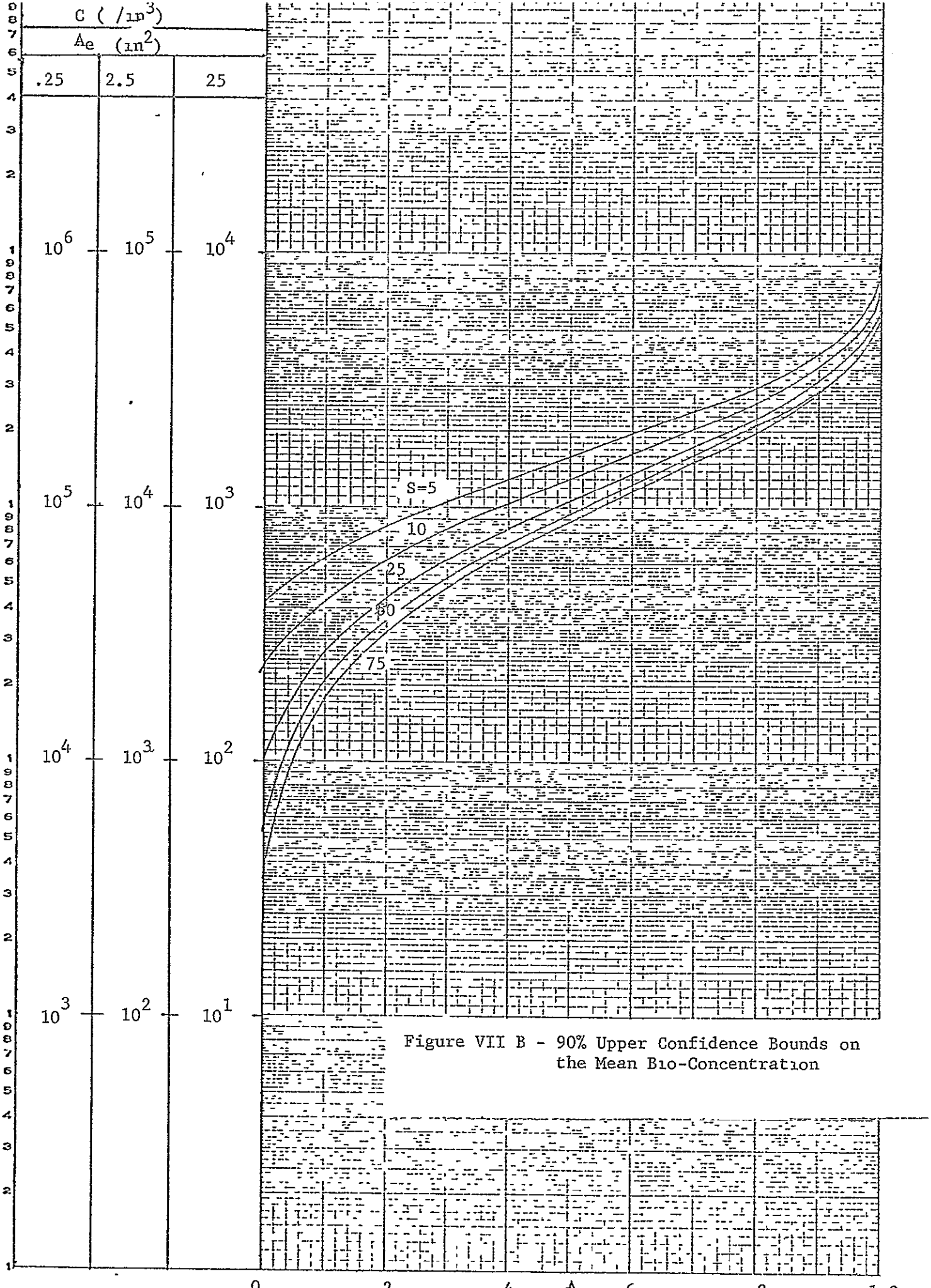


Figure VII B - 90% Upper Confidence Bounds on the Mean Bio-Concentration

REFERENCES

1. Schalkowsky, Samuel, Kline, Robert C , and DeGraff, Elliott Effect of Microbial Release Probabilities on Spacecraft Sterilization Requirements. Tech. Rept SR-034, Systems Res. Div., Exotech Inc , Aug 1968.

2. Fraser, S J. The Survival and Release of Viable Microorganisms from a Solid Material After a Hard Impact. Rev. Ltr. D2-114143-1, The Boeing Co. , n d

APPENDIX D

The Release of Buried Microbial Contamination by Aeolian Erosion

THE RELEASE OF BURIED MICROBIAL CONTAMINATION BY AEOLIAN EROSION

Prepared by

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J. Lyndon Woodall

under

Contract NASw-1734
National Aeronautics and Space Administration

August 1969

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The Release of Buried Microbial Contamination by Aeolian Erosion

A. INTRODUCTION

1. Brief statement of planetary quarantine objective
2. Description of the physical process treated

B THE EROSION PROCESS

1. Derivation of $\Delta V/V$ for a sphere
2. Numerical data for erosion rates of lucite, aluminum
3. Numerical data for Martian wind velocities

C. PROBABILITY OF RELEASE

1. Derivation of $P(\text{release} \mid \Delta V \text{ erodes in } t_q)$
2. Sensitivities of $P(\text{release} \mid \Delta V \text{ erodes in } t_q)$

D. CONCLUSIONS AND RECOMMENDATIONS

DESCRIPTION OF THE PROCESS BEING TREATED

The impact of a lander on a planet could have serious consequences if one desires not to contaminate the planet. In the light of this one should examine the implications of fracturing and exposing of surfaces which might "instantaneously" or subsequently release viable spores. Here we will consider the relatively slow process of erosion although the fracture and the erosion phases are not necessarily independent. For our purposes we will take the fracture as having occurred and characterized by a fracture-ratio. (ref. #2) The fracture-ratio is defined as the area exposed through fracture divided by the volume of the sample. An expression for the erosion of spherical shaped particles and an expression for the probability of release given that a quantity of the sample erodes in the quarantine period have been derived. Calculations based on experimental data have been made for the erosion rate.

THE EROSION PROCESS

Assume no preferred wind direction and assume the fragments roll about freely. Then erosion of a fragment by wind-borne agents is often radially symmetric. This suggests that we can study the erosion of a sphere as typical of the erosion process.

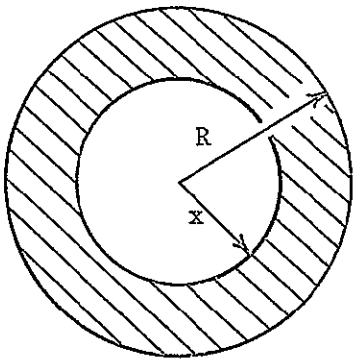


Figure 1. The geometry which will be considered here.

The ratio of volume removed to original volume of the fractured piece is

$$\frac{\Delta V}{V} = 1 - \left(\frac{x}{R} \right)^3 \qquad (1)$$

(case 1) $x = R - \epsilon tq$ if $R \geqq \epsilon tq$

or (case 2) $x = 0$ if $R < \epsilon tq$

where ϵ = erosion rate (rate of eroding surface) (ref. #2)

tq = time of quarantine.

when $R \geqq \epsilon tq$, Equation 1 becomes $\frac{\Delta V}{V} = 1 - \left(1 - \frac{\epsilon tq}{R} \right)^3$ (2)

The equation in this form is unmanagable due to the fact that the R parameter is still present. We would like to find R as a function of a macro-parameter, say the fracture-ratio f as previously defined (ref. #2). To do this it is necessary to assume some break-up model (i.e the way R is related to the dimensions of the original sample of material)

Suppose the original sample were a cube of edge length L, and fractures completely into equal sized particles of which a sphere of radius R is typical. The number of particles is approximately

$$N = \frac{3L^3}{4\pi R^3} \tag{3}$$

and the fracture ratio is $f = \frac{S_f - S_1}{V} = \frac{N4\pi R^2 - 6L^2}{L^3}$

where S_f is the final surface exposed and S_1 is the initial surface. Equation (3) simplified gives,

$$\frac{1}{R} = \frac{f}{3} + \frac{2}{L} \tag{4}$$

Equation (2) then becomes $\frac{\Delta V}{V} = 1 - \left[1 - \epsilon t q \left(\frac{f}{3} + \frac{2}{L} \right)\right]^3$. (5)

The erosion rate (ϵ) can be determined from existing data. In experiments by Neilson and Gilchrist (ref. #3), lucite and aluminum were eroded by aluminum oxide particles of 210 micron diameter. The results show that

$$\frac{dm}{dt} = E(\theta) \cdot \phi \cdot A \tag{6}$$

where $\frac{dm}{dt}$ = the rate of loss of mass

$E(\theta)$ = a proportionality factor depending upon the angle of attack and the material

ϕ = the mass-flux of particles per unit area striking the surface.

A = the cross-sectional area

The mass eroded from the sphere in Figure 1. is

$$\Delta m = d\Delta V = d\frac{4\pi}{3}(R^3 - x^3) \tag{7}$$

where d = density of substance being eroded

Differentiating (7) with respect to time and combining with (6) gives,

$$\epsilon = \frac{dx}{dt} = \frac{E(\theta) \cdot \phi}{2d} \tag{8}$$

Substituting equation 8 into equation 5 gives,

$$\frac{\Delta V}{V} = 1 - \left[1 - \frac{E(\theta) \cdot \phi t_q}{2d}\left(\frac{f}{3} + \frac{2}{L}\right)\right]^3 \tag{9}$$

ESTIMATION OF PARAMETERS

Hertzler at McDonnell Aircraft Corporation in simulation of the Martian atmosphere has arrived at estimates of velocities of winds on Mars and volume of abrasives carried by the wind (ref. 4)

Average wind velocity = 220 fps.

Particle concentration = 10^{-4} oz./ft^3

Then the mass-fluence per unit area is,

$$\phi = 4.68 \times 10^{5} \text{ lb/M}^2\text{/YR.}$$

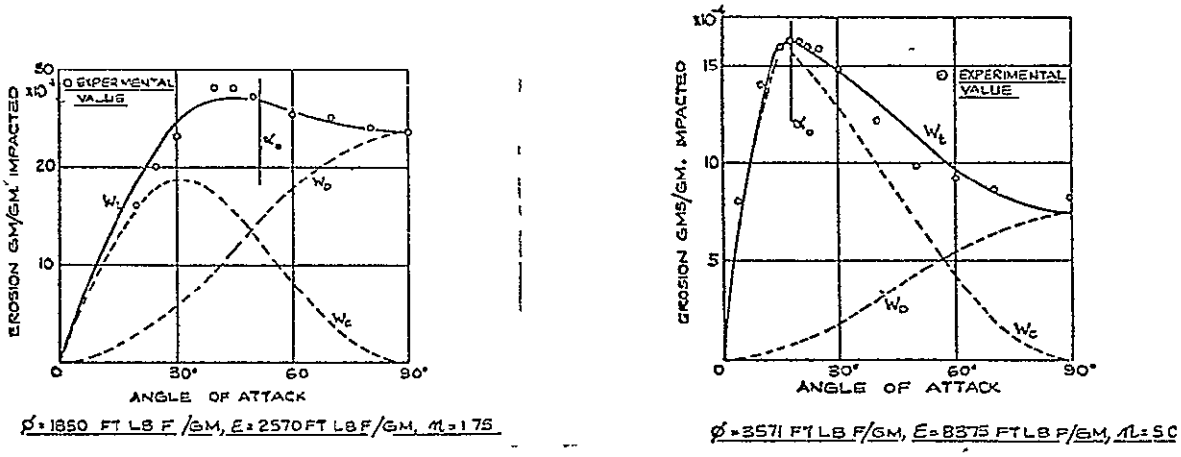


Figure 2. (after Neilson and Gilchrist) ref. #3

(a) Erosion vs. angle of attack characteristic for lucite eroded by 210μ aluminum oxide particles at 420 fps.

(b) Erosion vs. angle of attach characteristic for aluminum eroded by 210μ aluminum particles at 220 fps.

From figure 2a one may estimate E (ϕ). We will take the maximum of the curve for lucite for our calculation. (i.e. $E(\theta) = E = 2.5 \times 10^{-3}$)

Note, however, that the experiment for lucite was conducted for particles at 420 fps where as we are postulating wind and particle velocities averaging 220 fps. A corrected estimate of E is taken to be

$$E = 2.5 \times 10^{-3} \left(\frac{220}{420} \right)^2 = 6.9 \times 10^{-4}$$

For lucite $$d = \frac{1.3 \times 62.4}{(.305)^3} = 2.9 \times 10^3 \ \text{lb/M}^3$$

or the erosion rate for lucite is $$\epsilon = \frac{E \cdot \phi}{2d} = 5.6 \times 10^{-2} \ \text{M/YR.} \qquad (10)$$

A similar calculation for aluminum yields $\epsilon = 1.9 \times 10^{-2}$ M/YR.

MODEL OF THE PROBABILITY OF RELEASE

Assume a cube of edge length L impacts and fractures with fracture-ratio f. Also, assume that ΔV of the original volume V is eroded away during the period of time tq. If M spores are distributed randomly in the volume V then

$$P(\text{release} \mid \Delta V \text{ erodes in } tq) = 1 - e^{-M\frac{\Delta V}{V}} \qquad (11)$$

Substituting equation 10 into equation 9 and combining with equation 11 gives an estimate of the probability of release

$$P(\text{release} \mid \Delta V \text{ erodes in } tq) = 1 - e^{-M\left\{1-\left[1-\frac{E\emptyset tq}{2d}\left(\frac{f}{3}+\frac{2}{L}\right)\right]^3\right\}} \qquad (12)$$

SENSITIVITY OF P (re/ΔV erodes in tq) TO ϵ, f, AND L

We calculated the following two cases.

PARAMETER	BEST	WORST
ϵ	10^{-6} m/year	10^{-2} m/year
f	10^{2} m^{-1}	10^{5} m^{-1}
L	1 m	10^{-2} m

Graphs Figure 3a and 3b are the results of the calculations. In both graphs the model indicates virtually no dependence on L, the size of the sample, and only when all parameters are "near" best case does dependence on ϵ, f become important. In other words, the amount of material eroded from the spacecraft debris, after a hard impact, is almost independent of its size but does depend on the fracture ratio f due to impact, and the erosion rate ϵ of the materials present. A further observation can be made if the quantity

$$\epsilon tq \left(\frac{f}{3} + \frac{2}{L} \right) << 1$$

then Eq. 12 becomes $P (re/\Delta V) = 1 - e^{- M \epsilon tq (f + \frac{6}{L})}$ (13)

furthermore if $M \epsilon tq (f + \frac{6}{L}) << 1$

then expansion of the exponential gives the approximation $P (re/\Delta V) = M \epsilon tq (f + \frac{6}{L})$ (14)

Note the similarity between Eq. 14 and the model of $P(re/V_I)$ in reference No. 1 if one makes the following transformations

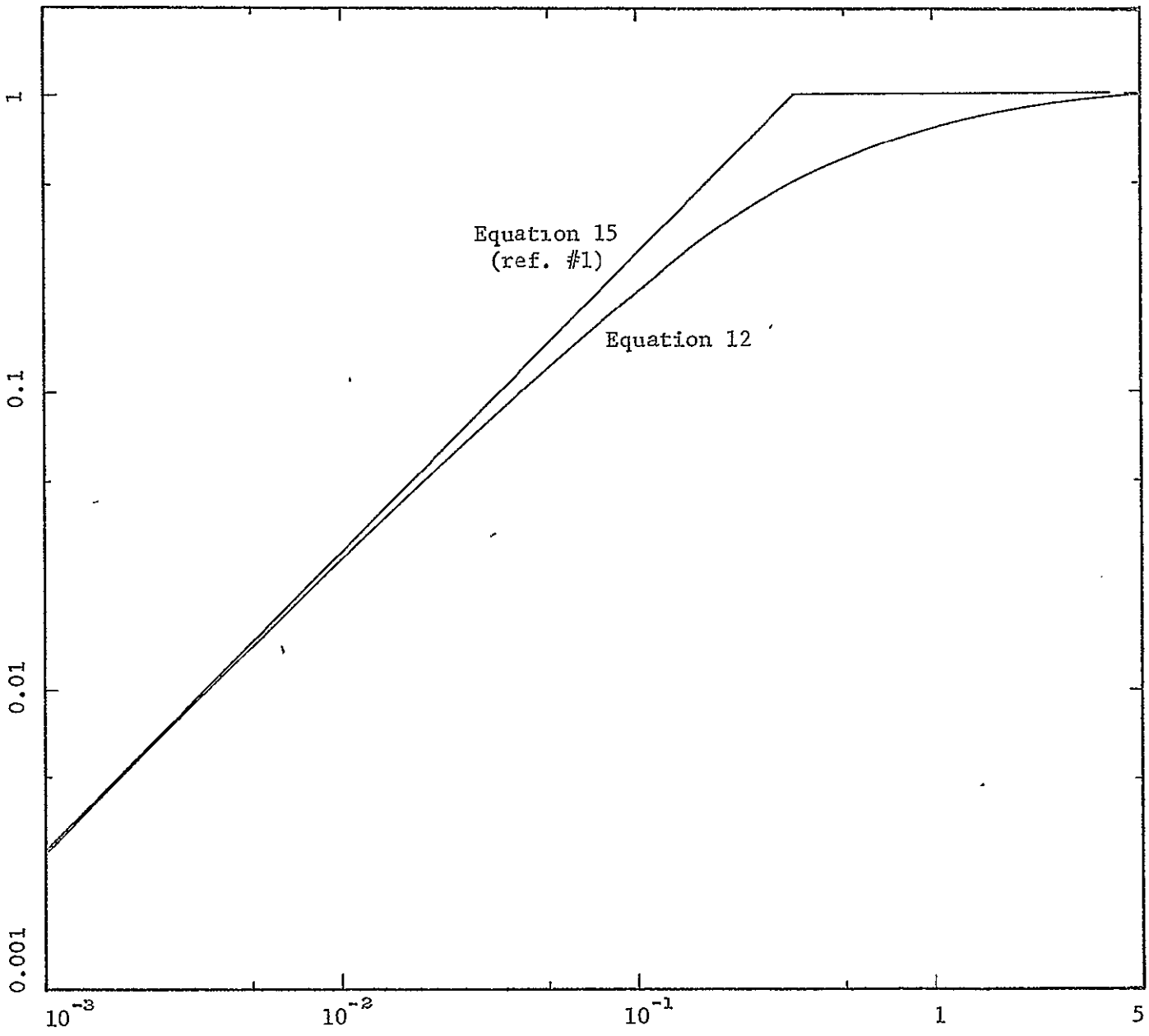
$$f^{(o)} \longrightarrow \frac{6}{L} = \frac{6L^2}{L^3}$$

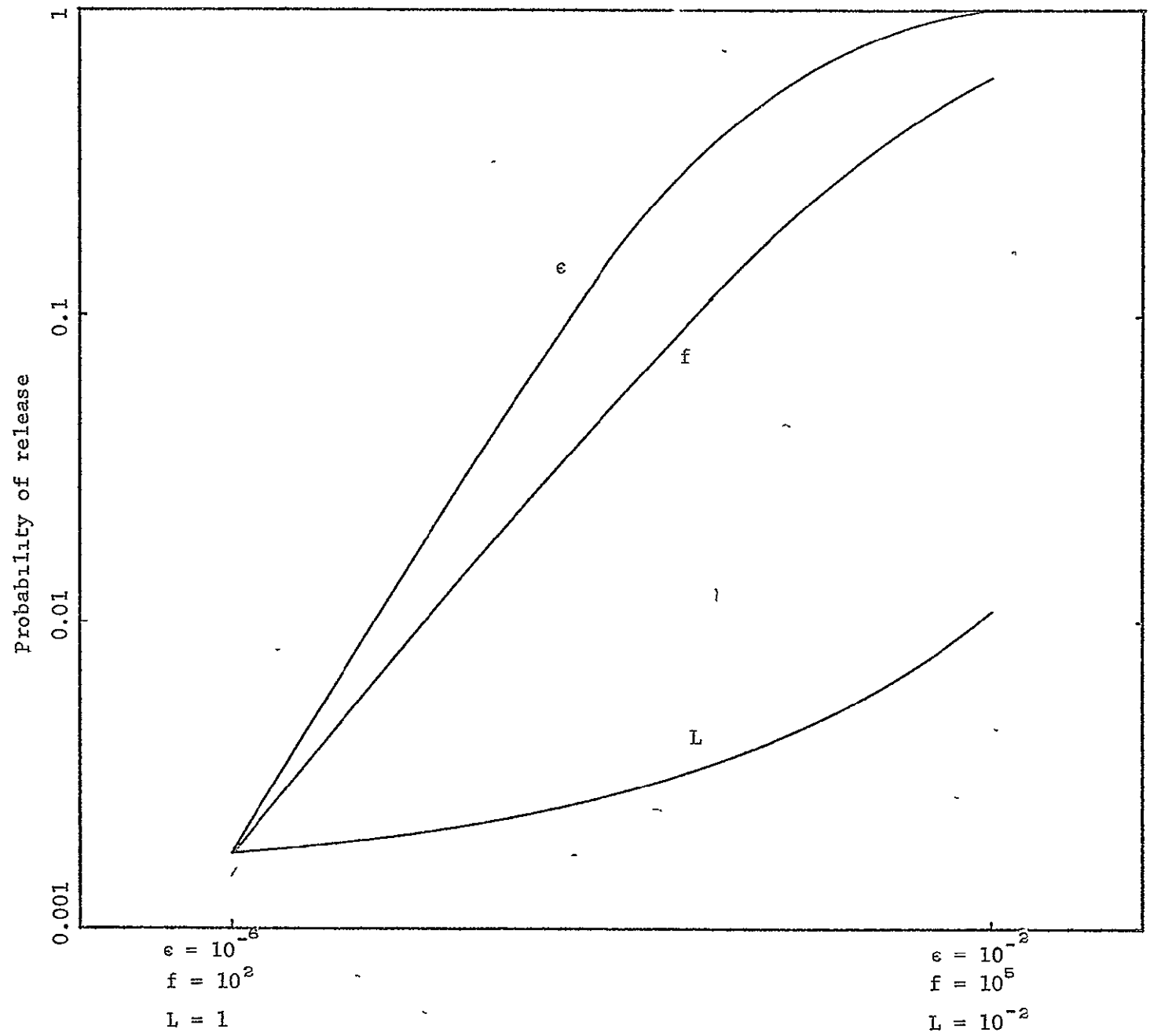
$$f \longrightarrow f$$

$$M(o) \longrightarrow M$$

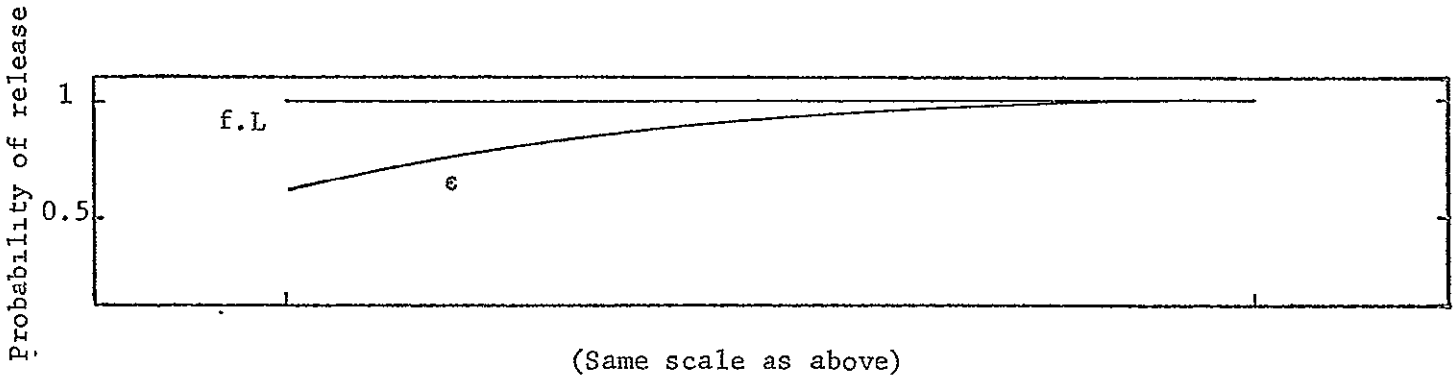
$$P(re|\Delta V) = M(o) \ \text{etq} \ (f^{(o)} + f) \qquad (15)$$

Figure 4 shows the error involved in using equation 15 instead of equation 12 as a function etq $(\frac{f}{3} + \frac{2}{L})$ if M is on the order of 1.


1
0.1
0.01
0.001
Equation 15
(ref. #1)
Equation 12
10^{-3}
10^{-2}
10^{-1}
1
5
$\epsilon\ t\ q\left(\frac{f}{3}+\frac{2}{L}\right)$



3a. All "best" case except for variation of one parameter



(Same scale as above)

3b. All "worst" case values except for variation of one parameter.

CONCLUSION AND RECOMMENDATIONS

Curves 3a and 3b indicates that the dimensions of the original sample are not of primary importance in estimating the P (release | ΔV erodes in tq) .

On the basis of this model the erosion rate ϵ and the fracture ratio (f) show no clear domination one over the other for the range of parameters considered. There is difficulty in estimating both but there is more uncertainty about ϵ. The range chosen for ϵ is 10^{-2} M/yr. to 10^{-6} M/yr. This involves 4 decades and includes the totally "worst case" situation in which the eroding agent is assumed to be uninterpreted at a rate of 220 feet per second for 17 years! The other extreme is the order of magnitude of terrestrially observed erosion rates. This wide range of uncertainty arises from our uncertainty about the flux of the eroding agent, $\emptyset$. It is recommended that a closer examination be taken of $\emptyset$ with a view to arriving at an estimate of the expected abrasive-flux. This might possibly be done by examining the distribution in crater populations of the moon and Mars as photographed by Mariner's V, and VI to arrive at a better estimate of $\emptyset$ for Mars. Because of the scarcity of small craters on Mars (Ref. #5) estimates of the extent of the transport-erosion process can be made.

REFERENCES

1. Exotech, Inc., Systems Research Division, "Development and Application of a System Model for Spacecraft Sterilization", Final Report Contract NASw-1558 for NASA, August 1969.

2. S. Schalkowsky, R. Kline, E. DeGraff, "Effect of Microbal Release Probabilities on Spacecraft Sterilization Requirments", Interim Report Contract NASw-1558 for NASSA, Office of Planetary Quarantine, August 1968, EXOTECH, INC.

3. Neilson and Gilchrist, "Erosion by a Stream of Solid Particles" WEAR Vol II 111-122 Feb. 1968.

4. G. Dyhouse, "Simulated Martian Sand and Dust Storms and Effects on Spacecraft Coatings", ASTM/IES/AIAA Second Space Simulation Conference (Sept. 1967). Am. Soc. Testing Materials, 1967 Philadelphia, Pennsylvania.

5. Leighton, et. al .. Mariner 6 and 7 Television Pictures Preliminary Analysis, Science Vol 166 No. 3901. Oct. 3, 1969.

APPENDIX E

Implementation of a Chemical Contaminant Inventory for Lunar Missions

IMPLEMENTATION OF A CHEMICAL CONTAMINANT INVENTORY FOR LUNAR MISSIONS

Prepared for

National Aeronautics and Space Administration
Office of Bioscience Programs

Contract NASw-1734

December 1969

by

EXOTECH INCORPORATED
Systems Research Division
525 School Street, S.W.
Washington,D.C. 20024

TRSR 70-07

INTRODUCTION AND SUMMARY

This report summarizes the study conducted by Exotech Incorporated under Task D of the subject contract. This task was initiated under modification No. 2 to the contract dated August 15, 1969.

The study described herein represents a follow-on effort to the planning study conducted by Exotech Incorporated under an earlier contract aimed at selecting an approach to establishing an organic constituent inventory for the Moon[1]. Based on the results of this planning study NASA selected an approach containing the following two essential guidelines:

(a) Documentation of lunar mission spacecraft should be preserved for possible future examination to identify types and quantities of organic materials deposited on the Moon. A detailed analysis of this documentation should not be undertaken until justified by requirements from investigation of lunar sample materials.

(b) An information system should be established which identifies regions of the Moon where the risk of contamination in surface samples is significant, including identification of the degree of risk and the spacecraft which contribute to the location - dependent material contamination.

The present study represents the initial step in the implementation of the above approach in that it considers the detailed procedures and tasks to be undertaken to collect, evaluate, store and disseminate data which will serve anticipated needs of lunar sample investigators, consistent with the requirement that costs associated with implementation and operation of the inventory be consistent with known needs for this information. The primary tasks pertinent to this effort involved (1) determining the availability of lunar mission vehicle documentation and the means for collecting it in a form suitable for future evaluation, (2) the collection and

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[1] Planning Study for an Organic Constituents Inventory Program, R.G. Lyle, Exotech Incorporated, Report No. TRSR-68-029 under Contract No. NASw-1666, May 1968

utilization of spacecraft trajectory parameters, landing sites and dispersion patterns for crash and soft landings, and (3) evaluating the compatibility of required date inputs with the existing Planetary Quarantine information system.

Sections II and III of this report summarize, respectively, the approach taken in this study and the detailed analysis of the questions considered. This is followed in Section IV by a set of recommendations for implementing the chemical materials inventory in accordance with the guidelines set forth above. A summary of these recommendations is also provided below.

The minimum requirements for the preservation of pertinent materials information are retention of documentation by lunar mission spacecraft contractors and notification of the Planetary Quarantine Office prior to disposal. This would apply to NASA contractors for the Ranger, Lunar Orbiter, AIMP, Surveyor and Apollo programs. In addition to this, however, the following procedures are recommended for consideration by NASA:

1. Designate a Federal Records Center to receive all documents and organize a filing system by mission designations.

2. Order all documentation sent by U.S. Mail to the designated Federal Records Center at end of the existing contractual requirements for retention, including those documents already in other records centers.

3. When contractors and agencies desire to retain copies of the information, require film copies suitable for aperture card insertion to be forwarded to the designated center.

4. Expand COMAT/TRIS System of Apollo program to include materials information on module sections not now covered. Information is not to include flammability or other data as in current system, but should include specification and property information which would be of use in identifying portions of the material at a later date.

5. Require copies of documentation on future flights to be supplied within thirty days after launch.

Concerning the preparation of an information system, a two step procedure is recommended as follows:

1. Prepare a contamination risk model of the lunar surface either in tabular form or preferably as a "risk-contour" map, based on the best available estimates of spacecraft landing sites and the particle dispersion associated with the mass and velocity characteristics of hard and soft landers. Dispersion models suitable for this purpose are the Sandia and Grumman models referred to in the text.

2. Disseminate among lunar sample investigators, and to others associated with the planning of lunar scientific exploration information concerning the documentation to be kept in storage and the available information. Include a questionnaire to permit the estimation of expected usage of information systems data of various levels of detail.

The above recommendations are expected to result in an optimum procedure for the implementation of a lunar chemical contaminant inventory, consistent with known cost and technical constraints. These recommendations and the source data upon which they are based, are elaborated upon in the body of this report.

The study described herein has been conducted by Robert G. Lyle and Lester D. Shubin of Exotech's Systems Research Division, under the overall direction of Samuel Schalkowsky. The contributions of highly pertinent information by the various personnel cited in this report from NASA, NASA contractors and from the scientific community, are gratefully acknowledged.

II. APPROACH TO THE STUDY

1. Definition of the Problem

The proper application of the results of this study should make possible the preservation of space vehicle documentation from which contaminant identification and quantization may be developed if required, and should enable the delineation of contamination risk zones on the lunar surface.

In order to gain an in-depth understanding of the entire problem, it was subdivided into separate tasks. These tasks define the inputs required to accomplish the goals set

a. Determine the location and form of the documentation required to identify spacecraft materials.

b. Examine the available dispersion models developed for high speed impact and soft landing vehicles and determine their applicability.

c. Determine the most productive course to follow in the collection and storage of pertinent documentation.

2. Procedures Followed in the Investigation

Personal communication was established with the cognizant personnel (see Table 1) at the agencies and companies holding the documentation. Discussions covered the availability of the documentation, the amount, the current form, its storage location, and the length of time that it would be retained. These conversations were followed by written correspondence which formalized estimates by these companies of the costs of copying, transferring and sorting the documentation in several ways. These alternatives are presented within this report.

Documents were acquired from the Grumman Aerospace Corp., detailing the dispersion model for contaminants emanating from soft landers and from the Sandia Corp., relating to both the dispersion of fragments from hard landers and the Planetary Quarantine Lunar Programs Information System. These documents were examined to ascertain the applicability of the dispersion

TABLE 1

PROJECT	NAME OF CONTACT	ORGANIZATION
Ranger	Mr. Irl Newland,* Librarian	JPL
Surveyor	C. W. Lefever, Mgr.** Contracts, SSD	Hughes Aircraft
Orbiter	H. M. Miller, Mgr. *** Contracts Space	Boeing
Apollo	Arlie Carter J. Steinthal	GE/Houston NASA/MSC
AIMP	F Ledoux	GSFC

*For Technical Information

* Mrs V. Pritchard (213-354-4321)

** Perry Ackerman (213-648-4134)

*** W. C. Galloway (206-656-2121)

models to the inventory problem and to define the interface between the existing information system and the inventory. In addition to this literature study, personal communication was established with a number of personnel concerned with the content of these documents, and with related problems of lunar surface contamination.

In addition to the personnel listed in Table 1, conversations were held with the following persons:

1. Mr. A.L. Roark of Sandia Laboratories on the Lunar Information System computer program;
2. Dr. M. Aronowitz of Grumman Aerospace Corp., on the dispersion model of the LM exhaust products;
3. Dr. A.L. Burlingame, Space Sciences Laboratory, Univ. of Calif., on the sensitivity of mass spectrometric measurements of lunar materials,
4. Dr. Elbert King, Curator of the Lunar Receiving Laboratory, concerning current practices of sample preservation,
5. Dr. P.R. Bell, Manager of the Lunar Receiving Laboratory, concerning possible need for the inventory,
6. Dr. Donald Flory, Gas Analysis Laboratory, LRL, to discuss LM exhaust products, and possible contamination in the Surveyor 3 crater;
7. Dr. I. Adler of the Theoretical Branch at GSFC, to explore possible needs of the inorganic chemistry investigators.

Communication was also established with the Data and Tracking Group at JPL to obtain the impact location of Orbiter IV, and with the National Geographic Society in order to determine the accuracy and sources of information used for placement of the impact sites of the USSR vehicles on their Moon chart.

The Micromation Co., of Washington, D.C. supplied estimates of the cost of various methods of copying documents.

When all of the information was at hand, the alternatives were studied and conclusions were reached concerning the procedures which would accomplish the intended goals in the most effective manner. Recommendations have been developed on the basis of these conclusions which will enable the cost effective implementation of the previously selected inventory approach, mentioned in the Introduction and described in detail in Exotech's previous report[2].

[2] Ibid.

III. ANALYSIS

A. Current Status of Documentation

Ranger: The current status of the Ranger Project documentation, the earliest of the programs under study for this report, has been reasonably well defined. All documentation currently existent is stored under the cognizance of Mr. Irl Newland, Chief Librarian, and his assistant, Mrs. Vivian Pritchard, of the Jet Propulsion Laboratory. The bulk of the material is stored at the Federal Records Center, at Shelly Air Force Base. The documentation is supposed to be reviewed every three years for possible disposal, but to date this has not been done, and the Exotech investigator was told that it will be stored indefinitely. Some documentation continues to turn up in various files in JPL offices and is sent to the library when it is found.

Most of the Ranger documentation is on 16 and 35mm film and much of it, especially the drawings, is stored on aperture cards for easy retrieval. Good estimates of the amount of documentation involved in the Ranger project have been difficult to obtain, but the best available indications are that approximately 10,000 documents and drawings are contained within the files.

Lunar Orbiter Boeing Aircraft Co., Seattle, Washington is contractually obligated to retain Lunar Orbiter spacecraft documentation for the following time limits: technical data-3 years, financial data-10 years. In each case the period begins from the time of the official termination of the contract. It should be noted that parts lists for the Orbiter spacecraft are contained within 13,000 drawings and identify the applicable specifications which are on file at the Department of Defense Information Center, where they are available to NASA.

Surveyor: The Surveyor spacecraft was fabricated in two configurations. Surveyors I through IV are designated Group A, Surveyors V through VII are Group B. There are sufficient differences between the two configurations to warrant this division, which results in an increase in the amount of documentation required to completely describe the spacecraft. The documentation system used by the contractor, Hughes Aircraft Company, permits reproduction of both sets of data.

The documents pertaining to the Surveyor vehicles are currently held by the Hughes Aircraft Co., El Segundo, California in the form of films, aperture cards, and paper copies. Most of the technical data is recorded and stored on aperture cards which are of the punched card type containing a microfilm insert, coded for retrieval with the document identification such as the drawing number. The coding can be extended at any time to include such other identification as is needed.

The Hughes Aircraft Co. estimates that it has approximately 3500 aperture cards in its possession relating to the Surveyor project. It is likely that some additional documentation exists that Hughes has not included, since this is a surprisingly low quantity in comparison with the Boeing Company estimate for the Lunar Orbiters. However, the additional number of documents is not expected to be large and the estimate by Hughes Company is considered acceptable for the purposes of this study.

Apollo. There are some 600 major contractors and numerous sub-contractors and suppliers for the Apollo program. They have produced thousands of drawings and documents, many of which are pertinent to the identification of chemical contaminants placed on the Moon.

Discussions with Mr. Gerald White at the Grumman Aerospace Corp., Bethpage, N.Y., revealed that the General Electric Corp. Houston office is the operating contractor of the COMAT System for NASA on the Apollo project. COMAT is an acronym for "Characteristics of Materials". The COMAT system is a computerized central data bank used in the recording and control of the use of non-metallic materials in the crew bays of the Apollo spacecraft. It is designed for the storage and retrieval of data on the use, status and characteristics of non-metallic materials considered for application in the manned spacecraft. The usage data consist of an accounting of materials in terms of their locations in the spacecraft and quantities and the functional requirements of their application. The status data consists of evaluation of the material safety and habitability in its application in terms of such parameters as combustion rate, fire point, odor, and carbon monoxide emission. The characteristics data include selected elements of flammability and outgassing test data.

The documentation used to supplement the COMAT data system is handled by the TRIS system (Test and Reliability Information System)[3]. This is a specialized document acquisition, storage and retrieval system, using automatic data processing to provide multiple listings and cost-indexing. TRIS acquires, microfilms, stores, retrieves and distributes documentation required to assist the reliability and quality control groups in their evaluation of certain parts, materials, and other hardware considered for use in the Apollo Program.

AIMP: An examination was made of the status of the documentation of AIMP D and E at the Goddard Space Flight Center. Mr. Frank LeDoux, of the Project Office, estimates that approximately two cubic feet of paper documentation including photographs exists. The documentation contains lists of materials, test results, and manufacturers specifications on much of the spacecraft materials. It is currently maintained in loose leaf folders and stored in a filing cabinet by Mr. LeDoux at GSFC.

The photographs are a necessary input to the inventory since they are keyed to the test programs and the identification of spacecraft parts would be difficult without them.

According to Mr. LeDoux, identification of materials used in experiment packages would not present a difficult problem. This is due to the fact that his records are virtually complete, and he knows the location of the additional data required.

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[3] General Electric, TRIS User's Manual, Houston, Texas

B. Alternative Methods for Preserving the Documentation

At this time, it is possible to retrieve the bulk of the documentation relating to the U.S. missions which have impacted or soft landed spacecraft on the Moon. However, it may be seen from Table 2 that the ends of the contractors' retention periods for the technical data are in the very near future. This imposes a constraint on the time available for implementation of the documentation collection required for the selected inventory model.

The alternatives available to the Planetary Quarantine Office under the specifications of alternative (d) of the previous Exotech report[4] requiring the preservation of documentation of all lunar contact missions are in increasing order of complexity.

1. Extend documentation retention agreements with the contractors pending a later decision

The simplest solution to the problem of preserving the documentation is to extend the retention agreements until at least 1975 and postpone a decision on the disposition of the documentation until then. This will gain time needed to determine the full extent of the requirements of the investigators.

The primary advantage of this alternative lies in its simplicity, and relatively low cost. The disadvantages are many, including the fact that the documentation is not readily available for examination by persons other than the contractors. In addition, it will be widely dispersed throughout the country, making it extremely difficult to gain any meaningful information from the documentation without considerable travel and time investment.

2. Direct contractors to transfer documentation to a designated Federal Records Center when the retention time expires

Under this alternative, the Government would require

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[4] Lyle, Loc. Cit.

TABLE 2

PROJECT	CUSTODIAN OF DOCUMENTATION	APPROXIMATE NUMBER OF DOCUMENTS	CONTRACTOR RETAINS DOCUMENTATION UNTIL
Ranger	JPL	10,000	1970 (?)
Surveyor	Hughes	3,500	1973**
Orbiter	Boeing	13,000	1977**
Apollo	GE/Houston	unknown	1975**
AIMP	GSFC	4,000	Indefinite Project continuing

* Analysis not needed-document call outs included

** Estimated close of contract

(?) Estimated

the contractors to transfer the documentation as it exists to a designated Federal Records Center at the termination of the retention period. The Planetary Quarantine Office would be notified of any impending changes in the location of the record material.

The advantage of this alternative is that it requires virtually no expenditure of funds. There are no storage or handling charges, and no transportation charge to NASA if the U.S. mails are used for shipping. In addition, the documentation eventually will all be located in a single storage center.

The main disadvantages are the time delay involved in transferring the documentation to the Government and in the variety of forms which must be handled.

3. Copy documentation in any form and send to designated Federal Records Center

Under this alternative, the contractors would submit duplicates of the documentation in whatever form is convenient to a selected Federal Records Center prior to a specified date.

The principal advantage gained is that the documentation will be transferred to a single Government operated facility at an early date, permitting greater accessibility than if kept only by the contractors.

The primary disadvantage of this alternative is that a variety of documentation forms must be dealt with. Retrieval of information from a collection of such diverse inputs would be difficult and costly.

4. Copy documentation into a suitable form for present and future use and store in a designated Federal Records Center

The significant gain in this alternative is the uniformity in the format of the stored documentation. The use of a

single type of copy will result in a time saving in handling the material during any subsequent search and retrieval over that in the other alternatives. The preferred format would be one which could be used in an aperture card at a later date if desired.

This procedure will cost more than the others initially, but may save money in the long run if information retrieval requests are expected. It is not necessary to process the data into a final form, only into a form which may later be processed into final retrieval form. This may be accomplished by converting the documentation into microfilm which could be entered into an aperture card system at a later date.

If the decision is made to store all lunar contact spacecraft documentation, a single Federal Records Center should be designated as the repository rather than utilizing the Federal Records Center nearest the present location of the documentation. If the U.S. Mail is used for shipment of the documents, the cost of transportation to NASA can be neglected. It would therefore be more beneficial to collect the documentation at a central point. From the point of view of convenience, this should be in or near the Manned Spacecraft Center, because the Apollo program is still in progress, and will be for some time, thus making it desirable to examine all the documents at this single location.

Consideration was given to the feasible methods of duplicating the documentation. The alternatives are

Microfilm,
Microfiche,
Paper copies,
Aperture cards, or
Computer tapes.

From the point of view of the initial cost, microfilm and microfiche are slightly more expensive than the hard copies. This is borne out by the Boeing Co. quotations for copying the Lunar Orbiter documents. The cost of supplying paper copies is approximately $3600 less than film copies. However, film copies are better suited to automated search and retrieval from the total inventory collection of documentation. If the documentation is stored on microfilm at the outset, considerable effort may be saved later in the event that in-depth searches must be made.

Many of the documents now stored are in the form of aperture cards. Aperture cards, while basically yielding the same information as the films and hard copies, possess an advantage over the other forms with the exception of computer tape, in that they are easily retrieved using a machine sorter. The principal drawback is the fact that the film size constraints require two cards for many drawings for complete coverage.

Storage of the documentation in the memory bank of a computer must be considered, since techniques exist for the reproduction of drawings, circuit diagrams and similar representations. The computer has not been considered as the prime storage and retrieval system for the Ranger, Orbiter and Surveyor, because the expense of conversion to this type of system is unwarranted at this time. If it were planned to convert the documentation of these missions to a form usable on a computer, considerable time would have to be devoted to programming and input. This goes beyond the requirements of the Space Science Board, and should not be done without certain knowledge that there will be sufficient demands on the system to justify additional expenditures.

C. Other Alternatives for Documentation Preservation

In the event that the demands from the investigators are sufficiently numerous, and require more information than is available under the intended inventory collection plan, it will become necessary to augment the system to enable retrieval of more detailed materials information. Therefore requests were made of the contractors for estimates of the cost of extracting the materials data and transferring it to NASA in a usable form. The costs indicated here reflect the fact that the selection would be done by personnel experienced with the projects, resulting in cost savings due to time lost by inexperienced personnel in learning about the projects.

The Hughes Aircraft Co. proposed that the following six tasks would be required to accomplish this selection for Group A and Group B Surveyor spacecraft:

1. Obtain a list of materials approved for Surveyor and identify those containing organic material.
2. Review Indentured Parts and Drawing Lists, for Groups A and B configured spacecraft, and identify those drawings potentially containing organic material.
3. Obtain Duplicate Aperture Cards (DACs) for each drawing. On the average, two (2) Duplicate Aperture Cards are required per drawing, since the area covered by a DAC "frame" is limited to 44 inches.
4. Review the DACs for organic material and identify the type and amount of material involved.
5. Compile a matrix of the amount of organic material by type and subsystem or control item.
6. Conduct a study to determine the amount of organic materials contained in the rocket engine products of combustion, which would remain on the lunar surface.

It is estimated that the above effort would require 17 man-months and $1500.00 in Other Direct Costs (materials and reproduction) The

estimated total fixed price for this job, including general and administrative expense and profit, is $53,300.

In the case of the Lunar Orbiter Spacecraft, the Boeing Co. personnel who are intimately familiar with the spacecraft are still available and could accomplish the following tasks:

1. Examine all drawings and documentation.
2. Determine the composition of the airborne hardware.
3. Furnish a report containing the classification of the organics and their approximate weight.

This effort would require six months of effort at a cost to the Government of $105,591.

Exotech has been unable to get a firm quote from JPL with respect to the Ranger documentation costs for similar efforts.

AIMP documentation is to a large extent already broken down in the manner described and little additional effort is required to maintain the documentation in this form.

The estimated cost of completing the materials documentation for the Apollo program, copying it into a suitable format and entering it into the current COMAT/TRIS System is $50-100,000 It should be noted that many thousands of documents exist in the hands of approximately 600 contractors, sub-contractors and other suppliers. The content of these documents in terms of organic materials is unknown at this time, and this uncertainty contributes significantly to the cost spread.

D. Dispersion Models

A survey of analytical methods for evaluating the dispersion of contaminants from hard impacts and soft landings was conducted during the period of this effort. This survey was carried out in order to determine the applicability of existing models to the determination of contamination risk areas, and the requirements for programming the models into the Interactive Computer Information System for Planetary Quarantine for Lunar

Programs. Two models[5,6] were found suitable for use in the predicting contaminant spread as a result of landings on the Moon's surface.

1. Soft Landings

The model by Aronowitz et al covers the chemical contamination of the lunar surface by LM exhaust during a soft landing. The total contaminant distribution is bifurcated into two phases of contamination a far field distribution and a near field distribution.

a. Far Field Distribution

The gas plume issuing from the LM descent rocket engine nozzle into the vacuum around the moon interacts with the lunar surface causing contamination of the surface. The rocket plume has two major flow regimes Adjacent to the nozzle exit there is a compressible continuum fluid flow regime, but as the gas continues to expand out from the nozzle the density decreases, and a free molecular flow, far field regime develops.

When the LM vehicle, in its landing trajectory, is at an appreciable altitude, only the fully developed far field of the exhaust plume intersects the moon. This interaction produces the far field contamination that has been analyzed and determined by assuming free-molecular point-source flow of the exhaust gas in the lunar gravitational force field.

Flow Model - The flow model thus consists of a moving, free molecular-flow point source in the lunar gravitational force field. The velocity of the gas

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5 Grumman Research Dept. Report RE-242 - Investigation of Lunar Surface Chemical Contamination by LM Descent Engine and Associated Equipment by L. Aronowitz et al.,March 1966.

6 Report SC-M-68-539 "The Chances of Retrieval of Viable Microorganisms Deposited on the Moon by Unmanned Lunar Probes", by Martin S. Tierney, Sandia Laboratories, Aug. 1968.

molecules flowing from the source is the vector sum of the velocity at which the source (LM) is moving and the source exhaust velocity. At ignition of the descent engine the LM velocity is approximately half the exhaust velocity and so must be included in the analysis. The random thermal velocity is considerably smaller. The molecules follow orbital trajectory flight paths that may intersect the spherical lunar surface where, as a first approximation, they can be assumed to be fully adsorbed.

Analysis for Contamination Calculation - The total far field contamination distribution on the lunar surface is obtained by integrating at each of a series of fixed lunar points the time history of contamination flux for the time period of the far field portion of the LM landing trajectory. The input data (LM position and velocity and the point source exhaust velocity and density factor distribution) are such that the integration must be done numerically by determining the flux at discrete times over the powered descent phase of the LM trajectory.

The principal equation in the flux calculation is the standard gravitational-force-field particle-trajectory equation that defines the flight path of a particle as a conic section. This equation is most easily solved in a spherical coordinate system with origin at the center of the Moon and with polar axis going through a known point on the trajectory.

To calculate the total contamination at a fixed point on the lunar surface, the particle trajectory equation must be applied repeatedly to the source

as it moves along the LM trajectory. The movement of the source means that the local coordinate system for the particle trajectories rotates relative to the fixed point. Furthermore, the particle trajectory equation does not explicitly determine which particle will land at the fixed point. To circumvent these difficulties, a different, indirect approach must be taken. Therefore, at a given time or, equivalent, for a given position of the source, the velocity that a particle must have at the source to intersect the fixed point is calculated and this velocity uniquely determines the particle flux at that point.

b. Near Field Distribution

The near field distribution is concerned with the study of lunar contamination by the LM rocket gases when the vehicle is close enough to the Moon such that a region of continuum fluid mechanics exists from the exhaust nozzle down to the lunar surface. This problem is considered as the near field erosion problem. For purposes of this study, erosion characteristics will not be considered in the determination of contamination. However, the program can be used to calculate the redeposited particle distribution on the surface and the associated temperature testing for a suspension model. A conclusion reached in the near field distribution research is that particles of 0.1 mm radius may fall as far as 130 meters from the rocket nozzle centerline.

c. Adsorption Estimation

Adsorption of Rocket Exhaust Gas on the Lunar Surface has been calculated using a solid lunar surface

model. Adsorption of the LM descent rocket exhaust gas on lunar surface material can introduce significant amounts of contaminants into the samples of the lunar surface that the Apollo astronauts will bring back to earth for scientific analysis. Discussed herein is a model used for quantitative calculations of the amount of rocket gas adsorbed on the lunar surface, and the subsequent desorption of these surface contaminants.

The model chosen for the lunar surface is a rough plane. This choice agrees well with the current knowledge of the lunar surface. The composition of the lunar surface material, in this model, was considered to be mainly metal silicates.

As the LM descends toward the touchdown site, gas molecules from the rocket exhaust will strike the lunar surface. While the LM altitude is above 100 or 200 feet, the molecules striking the surface are in the free molecular flow regime. At lower altitudes, the gas contacting the lunar surface in the vicinity of the LM is in the continuum flow regime. The formulation uses gas-dynamic equations appropriate to the continuum regime.

d. Computer Program Usability

The computer programs developed for the contamination distribution estimates for both Far and Near field distribution as well as the adsorption computations are operational on an IBM 7094 digital computer. It is reasonable to conclude that these programs have been written in FORTRAN, a widely used scientific computation language. It is felt

that with some modifications to these programs, conversion to the CDC 3100 may be possible.

2. Hard Landings

The Sandia report[7] describes the dispersonal of contaminants during hard landings. Two possibilities are modeled.

- Dispersion of lunar soil ejected by a spacecraft making a hard landing on the Moon.
- A range distribution for fragments of a spacecraft making a hard landing on the Moon.

The range distribution model can be used to determine the probability that beyond a given distance from impact point no fragmentation is expected to be found.

The assumptions under which the range distribution probabilities are derived should be noted.

- Impact of the lunar probe is normal to the Moon's surface.
- Fragments are ejected isotropically.
- Angle of ejection of a fragment is independent of the fragment mass and speed.

The soil ejection model was not used. Its output is given in surface density of crater ejecta per square kilometer rather than fragmentation distribution.

The interaction aspects of the Planetary Quarantine Lunar Programs Information System are not operational, however, the computational algorithms for the hard impact model are operational on the CDC 3100 computer. The soft landing model is operational on an IBM 7094 computer.

3. Applications of Dispersion Models

Table 3 gives a listing of the latest information available on landing sites, impact mass, velocity, and date of contact. The location of Orbiter IV, previously unreported, was

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[7] Tierney, Loc.Cit.

TABLE 3
SPACECRAFT LANDING COORDINATES

U S	LAT.	LONG.	AREA NAME/CRATER	IMPACT MASS/KG	SPEED KM/SEC	DATE OF CONTACT
Ranger 4	13.9°S	129.4°W	*	331	2.669	4/26/62
Ranger 6	9.3°N	21.4°E	Mare Tranquillitatis	365	2.66	2/2/64
Ranger 7	10.7°S	20.7°W	Mare Cognitum	366	2.616	7/31/64
Ranger 8	2.7195°N	24.6195°E	Mare Tranquillitatis	367	2.651	2/20/65
Ranger 9	12.9°S	2.4°W	Alphonsus	366	2.669	3/24/65
Surveyor 1	2.46°S	43.32°W	Oceanus Procellarum Flamsteed	270	3.96 m/sec	6/2/66
Surveyor 2	4±0.4°N	11±1.1°W	Sinus Aestuum	292	2.38 m/sec(?) crash	9/22/66
Surveyor 3	2.99-3.06°S	23.32-23.34°W	Oceanus Procellarum	285	Soft Landing	4/19/67
Surveyor 5	1.45°N	22.25°E	Mare Tranquillitatis	281	Soft Landing	9/11/67
Surveyor 6	0.46-0.51°N	1.37-1.39°W	Sinus Medii	282	Soft Landing	11/9/67
Surveyor 7	40.89°S	11.44°W	Tycho	284	Soft Landing	1/10/68
Surveyor 4	0.42°N	1.33°W	Sinus Medii	284	U	7/16/67
Orbiter 1	16.35°N	160.71°E	*	387	2.38	10/29/66
Orbiter 2	3.0°N	119.1°E	*	392	2.38	10/11/67
Orbiter 3	14.6°N	91.7°W	Einstein	387	2.38	10/9/67
Orbiter 4	0±10°	26±5°W	Lansberg	392	2.38	10/6/67
Orbiter 5	2.79°S	83.04°W	D'Alembert Mts. Schluter	392	2.38	1/31/68
Apollo 10 DS						
Apollo 11	0°41'15"N	23°26'E	Mare Tranquillitatis West Crater			7/20/69
Apollo 12	3°S	23.3°E	Oceanus Procellarum			
Apollo 13	6°S	17°W	Fra Mauro			
Apollo 14	0°36'S	32°43'E	Censorinus			
Apollo 15	22°12'N	29°20'E	Littrow			
Apollo 16	41°45'S	11°30'W	Tycho			
Apollo 17	13°45'N	56°W	Marius Hills			

(cont'd)

SPACECRAFT LANDING COORDINATES

U.S.	LAT.	LONG.	AREA NAME/CRATER	IMPACT MASS/KG	SPEED KM/SEC	DATE OF CONTACT
Apollo 18	25°9'N	49°30'W	Schröter's Valley			
Apollo 19	8°3'N	6°E	Hyginus			
USSR						
Luna 2	30.1°N	0.01°E	Mare Imbrium/Autolycus	390	2.38	9/14/59
Luna 5	1.5°S	25°W	Oceanus Procellarum Lansberg	1476	2.38	5/12/65
Luna 7	9.8°N	48.8°W	Oceanus Procellarum/Marius	1506	2.38	10/8/65
Luna 8	9.6°N	61.6°W	Oceanus Procellarum Galilei	1552	2.38	12/6/65
Luna 9	7.8°N	65°W	Oceanus Procellarum	1360	5.5-6.1 m/sec	2/3/66
Luna 13	18.5°N	62°W	Oceanus Procellarum	100	Soft Landing	12/24/66
Luna 15	U	U	Mare Crisium	U	1.3 Km/sec (?)	7/21/69

* Not named
U Unknown

supplied by the Data and Tracking Group at JPL. Luna 15 data are not listed, due to the fact that at the time of this report, Exotech has been unable to acquire any information other than the report made by the Jodrell Bank Observatory during the Apollo 11 flight.

The proposed dispersion models must be combined and programmed with terminal trajectory information and impact sites to answer queries such as these

- What is the probability of the presence of a fragment or fragments of a specified size or mass at a designated sampling site?
- Which mission(s) would be the principal contributor(s) to contamination in a particular lunar region?
- At given distances from the impacts, what size range of fragments is to be expected?
- What is the closest point of approach to a previously landed spacecraft where the probability of detectable contamination is less than a selected value?

In order to provide answers to these and similar questions, both models must be utilized in such a way as to maintain their separate identity, and allow their outputs to be supplementary or independent. In this manner, the exhaust components from soft landers and fragments from impacts can be reported as part of a total contamination picture or as separate constituents.

E. Scope of Materials Documentation

The analytical efforts of the Lunar Principal Investigators are frequently directed toward the identification of trace amounts of components. If the analysis reveals the presence of a substance such as sulfur or phosphorus, the investigator will be concerned as to whether it is of terrestrial or extraterrestrial origin. The investigator will have an

interference problem whether the sulfur comes from sodium sulfate or from a type of rubber that deteriorated on the lunar surface. After prolonged periods of time, it may be that the two materials are virtually indistinguishable from each other. It would be helpful to the investigator, in any case, to have an estimate of the probability that a certain amount of sulfur can be expected as a contaminant in the area of interest.

From the point of view of the life scientists, in contrast to that of the analytical chemist, noted above, the source of the contaminant is important. After some treatments, notably gas chromatography/mass spectrometry, the original composition is destroyed, and the inorganic ions are the same, regardless of the source. The phosphorous from a phospholipid is indistinguishable from that from a phosphate, after treatment nitrate nitrogen appears the same as that from an amine. With additional effort, differences can be identified, but the problem is obvious.

The information available from the inventory would be more comprehensive if the scope were broadened to include all non-metallic materials, especially those materials which include their composition elements of biological composition. This list should include those elements such as N, P, S, Na, K, Mg, Ca, Sr, F, Cl, Br, and I for example and any others which have been found necessary for terrestrial life and are present in non-metallic compounds.

F. Future Requirements

The compilation of a lunar inventory of possible surface contaminants is predicated upon the fact that future needs will require information concerning the identity of these materials. It is obvious that the spacecraft documentation preserved in its present status cannot answer these projected needs. An assessment of future requirements is needed, since any detailed characterization, indexing or categorization of the documentation beyond that of identification according to missions is unwarranted (except for Apollo) at this time unless a real demand is expected.

A program should be initiated which will enable the prediction of these needs and thereby permit planning to accommodate them. Certain assumptions have been made in this study concerning future needs, although

the information needed for valid estimates is not available at this time. The first assumption that must be considered is whether or not justification exists for expecting that this documentation will be requested. The rationale for saying that it will be requested is based upon the expectation that the next generation of analytical instrumentation will operate at increased sensitivity levels, i.e., where parts per billion sensitivities are now commonplace, parts per trillion are likely to be obtainable in the near future. If this is indeed the case, background levels will have to be examined very carefully, and this may lead to requests for more details on the materials which make up this background.

Along the same line of thought, a twenty year period recommended for retention of documentation is considered sufficient to cover any future need for the inventory.

The information requests received during this period will also establish whether or not more detailed procedures are needed for a storage and retrieval system to manage the information contained in the inventory.

G. Maintaining the Inventory

During the study, considerable difficulty was experienced in attempting to update the landing parameters and location coordinates for several of the missions. In order to maintain the inventory with the best available information, such data should be frequently reviewed and updated. At present, they are scattered among the agencies responsible for the programs, and become less reliable as the groups change. A central source is needed which can provide this type of information to interested personnel. Since much of this effort is directly related to the manned spacecraft program, MSC, Houston is the logical Center to set up and maintain all data on landing sites, impact characteristics, and other pertinent information on lunar mission hardware. An effort should continue to include all available information on USSR landers as well.

In describing the potential usefulness of the inventory, an incident of recent occurrence should be noted. No plans have been made to check the accuracy of the model formulated by Aronowitz for soft landers either

by analyzing specimens taken near the LM on Apollo 11 and 12, or those taken from the crater containing Surveyor 3. A recent publication describing the LM exhaust products has been published[7], and has apparently enabled one researcher to identify a fluorescent material as having originated in the LM exhaust. Considerable benefit would result from verification of this model enabling the prediction of uncontaminated sampling sites, and in the degree of contamination to be expected close to the LM.

The only discrimination of classes of materials presently available in the inventory documentation is that of the Apollo program which separates out the non-metallic components. This is the only limitation applied to the collection of materials information and is a more realistic distinction than organic/inorganic. By increasing the prescribed scope of the inventory documentation to this extent, possibilities of omission would be decreased considerably at little cost since the Apollo documentation system is already observing this division. If this concept is adopted, the inventory would be capable of providing more comprehensive information than would be possible with an arbitrary elimination of inorganic materials.

Additional useful information which could be entered into the Planetary Quarantine computed-based information system includes the locations of the various sub-sections within the spacecraft documentation and general cross-reference index. To illustrate· Although the spacecraft materials information will not be analyzed or categorized, the primary index titles as utilized by the various contractors in filing the information could be carried through. Requests for information relating to a particular subassembly on Ranger VII could also provide file locations on similar subassemblies on Rangers VIII, IX, IV and VI at the same time. If information concerning the desired mission is incomplete similar data for an identical spacecraft could be indicated.

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[8] Apollo Lunar Module Engine Exhaust Products, B.R. Simoneit,A.L. Burlingame, D.A. Flory and I.D. Smith,Science, Vol.,166, No.3906, Nov. 7, 1969

IV. RECOMMENDATIONS

It is convenient and useful to present recommendations separately for (1) the preservation of documents and, (2) the implementation of a materials information system. The first category deals largely with detailed data on types and quantities of materials contained on particular flight vehicles for which there may or may not be a demand. The second category represents the general methods for providing to potential users information on the risk or likelihood of sample contamination and, if appropriate, also on the type, quantity and source of the contamination. The documentation category is therefore a subelement of the information system. However, since action taken on the preservation of documents - and more significantly, the lack of such action has an irreversible effect on the future usefulness of an information system, the recommendations concerning the preservation of documents require early attention.

A. Implementation of an Information System

To assure that costs are compatible with known benefits, it is recommended that implementation of an information system be carried out in two steps as follows:

1. Preparation of Contamination Risk Model for the Moon

Information concerning the likelihood of sample contamination by materials of terrestrial origin (due to prior lunar flights) should be prepared and made available to interested personnel within NASA and the scientific community. This is most readily accomplished either in tabular form or as a map of pertinent regions of the Moon containing contamination probability "contours".

The updating of the system at KSC with all pertinent lunar mission spacecraft parameters is required. The information is currently not available from a single source, but must be sought from the various responsible agencies.

The dispersion models of Aronwitz and Tierney should be programmed into the Planetary Quarantine computer system

in a manner that will provide identification of surface areas in the vicinity of spacecraft landing sites where contamination may affect the samples. In order to apply the dispersion models delineated by Aronowitz and Tierney, and to specify their effect on the various sampling sites, the best available information on the impact parameters and locations of the spacecraft must be applied. This input to the inventory should be updated as better information becomes available. It should be noted that the inclusion of this input to the information system is important in order to provide estimates of the distribution of contaminants over the lunar surface, and to provide an estimate of the probability of a contaminated surface sample being drawn from a particular region of the Moon. The more precise these inputs are, the better the estimates will be. Unlike the materials documentation input which treats U.S. spacecraft alone, this information treats all spacecraft, including those originating in the USSR.

The most important output from this system, initially, is the delineation of the contamination risk areas. The intended Apollo landing sites through Apollo 20 have been tentatively selected. Issuance of the expected contamination levels (risk areas) in these and adjacent areas would be valuable aids in planning these missions.

2. Assessment of Future Requirements

With the information now available, the precise questions to be expected from investigators of lunar sample materials cannot be predicted at this time with sufficient certainty. Provisions for gaining additional information on potential requirements are therefore necessary.

It is recommended that a questionnaire be distributed to recipients of the Lunar Contamination Model. The questionnaire should determine the aim of the research, the general

techniques and instrumentation to be used, the elements of interest, the interferences, the expected use of the data system, particular types of information desired, and other relevant data. From these questionnaires, estimated usages can be made, conclusions as to indexing requirements can be drawn and the type of retrieval system which will be most useful can be selected.

B. Preservation of Documents

1. Minimum Requirements

The fundamental consideration with respect to spacecraft materials documentation is that disposal or loss of pertinent records be prevented so long as a possible need exists for such information to support lunar sample investigations. The mechanism for preservation of these documents exists in the retention time requirements imposed upon the various contractors. This should be supplemented by a requirement for notification of the Planetary Quarantine Office prior to disposal or transfer of the documents from their present locations. Implementation of a notification requirement would ensure against inadvertent loss of the information.

2. Recommended Storage Procedures

A more direct approach to the solution of the problem is to order transferral of the documentation, or suitable copies thereof (see below), to the Federal Records Center nearest the cognizant agency at the end of the contractually specified retention time for technical data. The exception is the Apollo documentation. In this area, the COMAT/TRIS system should be expanded to contain the additional documentation required for all nonhabitat areas, e.g., the descent stage. The documentation need not be completely detailed with respect to test results of flammability or tests of other types pertinent to the current needs of the

system, but should contain all nonmetallic organic materials information pertaining to amounts, classifications, and specifications of the material. Incoming documentation should be separated into two categories: (1) that which contains no materials information; (2) the remainder. Category (2) should be indexed with a brief descriptive title and entered into the system. The responsibility for locating and transferring the documentation should be shared by the personnel currently managing the system, aided by the prime contractors, North American Rockwell, Grumman Aircraft and the Manned Spacecraft Center. The urgency of this move cannot be overstressed since construction of the remaining Apollo spacecraft is now virtually complete and personnel now assigned to these projects are likely to be transferred. With this action, the location of many documents in the hands of suppliers will become obscured. The documents pertaining to the habitability areas of the CM and LM are necessary, but in themselves not sufficient for the inventory, because the descent stage of Apollo 10 will impact at a future date, and the ascent stage from Apollo 12 impacted on November 20, 1969.

3. Suitable Copy Recommendations

Of the formats described previously, the one that is most suitable for both present and evisioned future needs is the aperture card. It is readily indexed in any way desired, lends itself to rapid and selective retrieval, requires little storage space in relation to the amount of information it can carry and is durable enough to withstand a great deal of handling. It is therefore recommended that the aperture card be established as the standard copy for all information to be copied for transferral, and that this also be the form that any future indexing or categorization should follow.

A requirement should be issued that the documentation concerning nonmetallic materials, their specifications, and other pertinent data, but excluding such items as flammability tests, for all future lunar missions be sent to the COMAT system for inclusion into the inventory. This will aid in keeping future search costs down. It is likely that little further documentation can be expected under the Apollo requirements except for the Lunar Vehicle, but this will constitute a large amount of material input that will not have to be processed at a later date. This can also accommodate the inputs from the possible lunar orbit shuttle vehicles for extended exploration of the surface.

In the event that requests from principal investigators warrant the additional effort necessary to provide more specific information concerning the organic materials, it is recommended that the COMAT/TRIS system format be used as a nucleus for an active inventory system.

ANALYTICAL TECHNIQUES IN PLANETARY QUARANTINE

FINAL REPORT
Contract NASw-1734

for

Headquarters
National Aeronautics & Space Administration
Planetary Quarantine Office

by

EXOTECH INCORPORATED
525 School Street, S.W.
Washington, D C. 20024

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TRSR 70-13

TABLE OF CONTENTS

INTRODUCTION AND SUMMARY

Contract NASw-1734 was initiated 23 April 1968. It specified analysis and planning activities to support NASA's Planetary Quarantine (PQ) Office in four areas, viz.

- Review and Interpretation of Planetary Quarantine Requirements
- Analysis of Microbial Survival
- Development and Application of Pertinent Analytical Techniques
- Implementation of a Lunar Chemical Contamination Inventory

The results of the work in these areas is reported herein Where preliminary findings have already been presented in interim reports, these are referenced Copies of these interim documents are appended in those cases where their content is felt necessary to support the material presented herein.

A WORK ELEMENTS

A listing follows of the work elements involved in the four tasks enumerated above

(1) Review and Interpretation of Planetary Quarantine Requirements

- Development of an approach to the justification of modified PQ requirements
- Review of PQ requirements for proposed Planetary Explorer Missions to Mars and Venus.
- Review of Mars Mariner '69 PQ Plan
- Review of Viking Project PQ Provisions document.
- Development of plans for the review of Viking PQ related documents.

(2) Analysis of Microbial Survival

- Survival curve analysis.
- Study of physical processes in microbial resistance to sterilization.

(3) Development and Analysis of Pertinent Analytical Techniques

- Analytical estimation of buried contamination
- Study of microbial release through material break up and erosion.

(4) Planetary Chemical Contamination

- Planning for the implementation of a Chemical contamination inventory.

B. DELIVERED PRODUCTS

Among the products delivered under this contract are

- Exotech Inc., Systems Res. Div.: An Analytical Basis for Assaying Buried Biological Contamination. Appendix C. Report no. TRSR-036. Jan. 1969.
- Exotech Inc., Systems Res. Div.: Implementation of a Chemical Contaminant Inventory for Lunar Missions. Appendix E. Report TRSR 70-07. Dec. 1969.
- Barrett, M. J.· Investigations into a Diffusion Model of Dry Heat Sterilization. Appendix B. Report no. TRSR-041, Systems Res. Div., Exotech Inc., May 5, 1969.
- DeGraff, E.. Review of JPL Report 605-87, Mariner Mars 1969 PQ Plan. Systems Res. Div., Exotech Inc., Memo., Aug. 21, 1968.
- NASA Headquarters Review of Viking PQ Plans. A Document Providing Background Information for Reviewers of Viking Plans.
- Space Bioscience Research material dated Nov. 5, 1969 for Code SB Document on Applications.
- Proposed Planetary Chemical Contamination Requirements statement, Feb. 1969.
- Guidelines for Review of JPL-Martin Bioburden Accumulation Model, Nov. 13, 1969.
- Exotech Inc., Systems Res. Div · Monthly Letter Status Reports as Required by the Contract.

C. PRESENTED PAPERS

- "Potential Effects of Recent Findings on Spacecraft Sterilization Requirements", presented at the 11th Plenary Meeting of COSPAR, Tokyo, Japan, May 14, 1968.
- Report on "Development of Analytical Techniques in Planetary Quarantine", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, June 10, 11, 12, 1968.

- "Effects of Mated D-Values on Terminal Sterilization Cycle", presented to Subcommittee 1A of PQAC, University of Minnesota, July 25 - 26, 1968.
- "Stochastic Diffusion Model for Microbial Survival in Heat Sterilization", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969.
- "Systems Approach to Compliance with PQ Requirements", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969.
- "Estimation of the Mean Concentration of Microorganisms Buried in Spacecraft Materials", presented at Semi-Annual Planetary Quarantine Seminar, Cape Kennedy, February 11 - 12, 1969
- "Tradeoff Studies in Heat Sterilization", presented to a committee of the Space Science Board at Stanford University on April 19, 1969.
- "Consequences of Stochastic Diffusion of Moisture in Microbes", presented at Semi-Annual Planetary Quarantine Seminar, Las Vegas, September 24 - 25, 1969.
- "Application of a Systems Model for Spacecraft Sterilization", presented at Semi-Annual Planetary Quarantine Seminar, Las Vegas, September 24 - 25, 1969.

D. OTHER MEETINGS ATTENDED

Additional meetings attended include:

- PQAC, NASA/Hdgts., October 1968.
- Microbiological Assay Standardization Meeting, NASA/Hdqts., December 13, 1968.
- Planetary Explorer PQ Meeting, NASA/Hdqts , January 29, 1969.
- Taft Health Center, Cincinnati, Ohio, April 22, 1969 - Review of Experimental Data on Microbial Die Off.
- Lunar Sample Analysis Symposium, 157th National Meeting American Chemical Society, Minneapolis, Minnesota, April 13 - 18, 1969.
- Viking Planetary Quarantine Review Meeting, Martin Co., Denver, Colorado, October 22, 1969.

II. RESULTS

Because extensive material has already been reported in interim documents and papers, those results will not be duplicated in this final report Where previous findings are felt necessary for the reader, they are provided in summarized form or appended

The material presented herein extends and complements this previous work. It is reported in the sequence of the four tasks enumerated in the previous section.

A. REVIEW AND INTERPRETATION OF PQ REQUIREMENTS

The pre-contract status of planetary quarantine standards, their formulation and their impact upon spacecraft programs was summarized in a paper[1] presented at the 11th Plenary Meeting of COSPAR on May 11, 1968, in Tokyo, Japan.

Although the work summarized at COSPAR was supported under earlier contracts (NASw-1558 and NASw-1666), a copy of the paper is attached (Appendix A) because it adds to the prospective of the work reported herein. The formulation of planetary quarantine standards was shown to be a continuing effort requiring a compromise between the assurance of the prevention of planetary contamination and the minimization of the impact of quarantine requirement on planetary missions. It was suggested that further work in several areas could lead to less severe sterilization requirements for planetary spacecraft than had been considered necessary in the past. Such areas for further research include tasks undertaken in the study reported herein. Of specific interest in this regard are·

- Estimation of buried contamination.
- Analysis of release of buried contamination.
- Microbial resistance to sterilization processes

Work in these areas is reported in Tasks B and C.

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[1] Exotech Incorporated, Potential Effects of Recent Findings on Spacecraft Sterilization Requirements, May 1968, also Space Life Sciences 1 (4) March (1969) 520-530.

The focus of activity in this initial task of contract NASw-1734 was in the application of planetary quarantine requirements. This is typified in the review of PQ constraints conducted in January 1969 for two proposed Planetary Explorer missions to orbit Venus and Mars. Probabilities of contamination for the two missions were computed based upon COSPAR constraints and estimates made by NASA's Planetary Program Office of the total number of different missions to be conducted within the quarantine period by all nations. The characteristics of the proposed Planetary Explorer missions were reviewed in the light of these PQ requirements. An analysis[2] performed by Bird Engineering Research Associates Inc. of the probability of contamination for the two missions was reviewed for accuracy and completeness.

Spacecraft project plans and provisions for compliance with PQ requirements were studied. In particular, the Planetary Quarantine Plan proposed for Mars Mariner 1969 was assessed.[3] The Viking project PQ Provisions document[4] was reviewed for compliance with PQ standards. In addition, plans were developed for the systematic review of further Viking project PQ related documents including:

- The PQ Plan
- The Microbiological Assay and Monitoring Plan
- The Sterilization Plan

Methodology for the development and evaluation of feasible alternative approaches for compliance with PQ constraints was presented in the Semi-Annual Planetary Quarantine Seminar at Cape Kennedy in February 1969.

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[2]Letter report 24 February 1969, Bird Engineering Research Associates Inc. to GSFC, Code 724 "Preliminary Report on Planetary Explorer Contamination Problem."

[3]Exotech letter 21 August 1968, subject. Review of JPL Report 605-87

[4]Langley Research Center document, "Viking Planetary Quarantine Provisions", No. M73-109-0, February 20, 1969.

B. ANALYSIS OF MICROBIAL SURVIVAL

Knowledge of the life and death processes of microorganisms is essential to the development of realistic PQ requirements and NASA's Planetary Quarantine Office has directed several research efforts in a total program with the objective of expanding this knowledge Exotech's efforts in this program involved a study of microbial resistance to sterilization. An earlier report[5] had suggested the advantages of log-normal over the simpler logarithmic model to describe the decay of viable microorganisms when subjected to heat sterilization This report recommended that further analytical work be complemented by physical modeling and be closely related to measured laboratory parameters.

The exceptional resistance of microbial spores to sterilization by heat had long been observed in laboratory tests The lack of a cohesive theory to explain this behavior, however, represented a weakness in the development of a defensible basis for the formulation of sterilization requirements. Exotech attempted under this contract to form an empirical framework from fragmentary theories and hypotheses so as to relate the effectiveness of environmental factors on dry heat sterilization as practiced in the NASA planetary quarantine program.

Earlier work reviewed included the "water activity" theory of Murrell and Scott[6] who observed experimentally the existence of an optimum water content in the spore that maximizes spore resistances to heat destruction, water and heat effect measurements by Angelotti[7], and more recent research

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[5] Schalkowsky & Wiederkehr "Estimation of Microbial Survival in Heat Sterilization" COSPAR Technique Manual No. 4, November 1968.

[6] W. G. Murrell and W. J. Scott, "The Heat Resistance of Bacterial Spores at Various Water Activities", J. Gen Microbiol. 43, 411-425 (1966).

[7] R. Angelotti, "Protective Mechanisms Affecting Dry-Heat Sterilization", COSPAR Technique Manual Series, Manual No. 4, November 1968.

of water intake and release by Campbell and coworkers[8]. Various mechanisms have been considered in an effort to explain this often-observed non-logarithmic heat destruction characteristic of spores in terms of their water content and its changes.

In the model under investigation the heat resistance of spores is attributed to the partial dehydration of some unidentified but vital protein in the cytoplasm. During sporulation, chemical binding of water to this protein is inhibited by the chelating agent, calcium dipicolinate. This action frees nonessential water molecules, which can then be squeezed from the cell by the surrounding cortex that appears to contract as the spore forms. During wet heat sterilization, water diffuses through the outer layers of the spore and interchanges with the chelating agent, thus, increasing the susceptability of the vital protein to denaturation, resulting in nonviable spores. The large differences between resistances of species may be due to the variable efficiency of the outer layers in inhibiting water diffusion. The great difference in heat resistance of B subtilis and B. stearothermophilus in aqueous solutions, which essentially disappears in dry heat sterilization, can be attributed to this diffusion effect

A time dependence of the spore's water activity can be predicted on the basis of a diffusion mechanism whereby loosely bound water migrates from one molecule in the microbe to another, with a mobility determined by the temperature and the existing gradient, according to well-known physical laws of diffusion.

A technical report was presented at the February 1969 Semi-Annual Planetary Quarantine Seminar at Cape Kennedy and early results were submitted in Exotech report TRSR-041, "Investigations into a Diffusion Model of Dry Heat Sterilization", May 1969, attached as Appendix B. Further results on this effort were reported at the Semi-Annual Planetary Quarantine Seminar in Las Vegas in September 1969. At that time the existence of two

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[8] Private communication, J. E Campbell, PHS, Cincinnati, Ohio.

distinct bonding sites for water in B. subtilis was demonstrated from data[9] supplied by Campbell. A sample of the evidence presented for this conclusion is given in Figure 1, which is a replot of the experimental measurements, demonstrating that the rate of water release can be described by the sum of two exponentials. This supports the theoretical framework of the diffusion approach, but further work would be necessary to identify and locate the two sites within the spore.

The implications of this work to the quarantine program are significant. First, it promises an analytical basis for the observed deficiencies in the simple logarithmic description of the microorganism die-off curve. The existence of a significant "tail off" in this curve can perhaps be quantitatively defined and predicted, thus, providing a defensible basis for the design of realistic sterilization cycles. This work also suggests the advisability of monitoring or perhaps controlling the humidity levels during assembly of spacecraft to modify the contaminants present for greatest susceptibility to subsequent sterilization conditions. Perhaps a low temperature preconditioning cycle during which the water activity is equilibrated at some sensitive value could precede high temperature sterilization to make it more effective. Finally, water content of such materials as lucite and epoxy might be controlled during manufacture to make their buried contaminants more susceptible to sterilization.

C. DEVELOPMENT AND APPLICATION OF PERTINENT ANALYTICAL TECHNIQUES

The need for improved estimation of the amount of buried contamination and of its probability of release was indicated in earlier work (see page 2) as worthy of further study. The difficulties in detecting and enumerating buried contamination derive from their inaccessibility. To overcome this, the contaminated material may be physically shattered or chemically dissolved and the resultant released microorganisms then counted. In both cases, however, there are serious questions of the validity of the yield since die-off can occur in the release processes.

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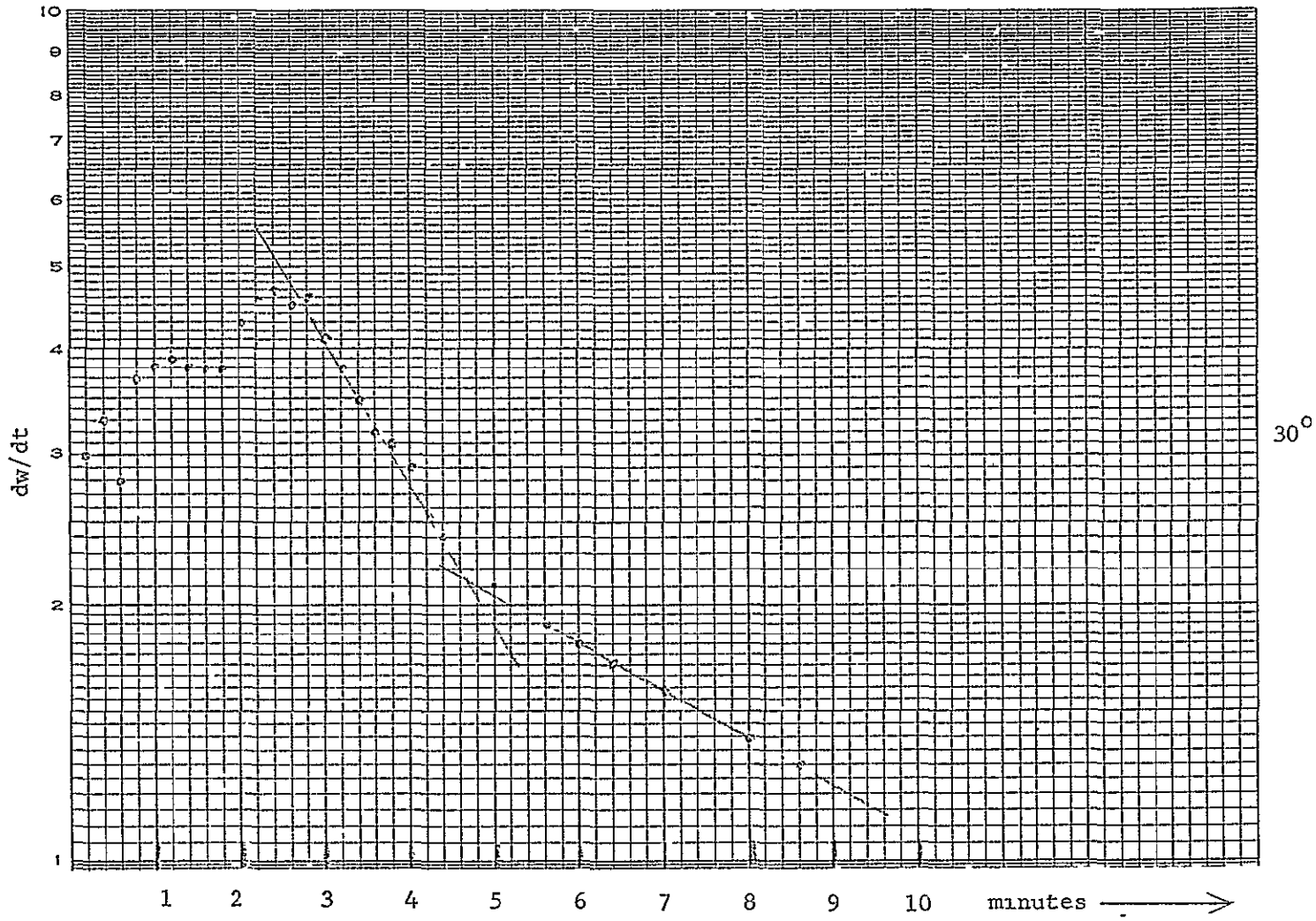
[9] Private communication, J. E. Campbell, PHS, Cincinnati, Ohio.

FIGURE 1
RATE OF WATER RELEASE AT 30°



Rate (mg/sec) at which water is released by a sample of B. subtilis spores in a current of dry air at 30°C. (Datus points, provided by Dr. J. E. Campbell of the Taft Health Center, Cincinnati, Ohio, have been corrected for background water release. Straight lines indicate that the sum of two exponentials would reproduce measurements after an initial build-up period.)

A statistical assay technique originally suggested by Mr. L. Hall, NASA Planetary Quarantine Officer, was further developed by Exotech and a protocol[10] proposed for its application (See Appendix C). The statistical assay technique does not require that all microorganisms contained in the material be recovered but produces an upper bound estimate based upon assay results of new surface exposed by minor fracturing which introduces minimum lethality.

The procedure requires the controlled fracture of representative samples of a material whose buried loading is of interest. Each sample is tested for biological contamination on the totality of new surfaces exposed as a result of the fracturing process The basic datum or observation consists of the proportion of samples which yield contamination upon culturing. Conventional statistical techniques, combined with an analytical expression relating the mean concentration of organisms buried within the material and the observed datum, produce an upper bound estimate for the unknown mean concentration, expressed to any prescribed level of confidence. In principle, the "confidence level" of the resulting estimate is directly related to the sample size and the amount of surface area exposed by fracture, as the sample size and exposed area increase, the difference between the estimate and the unknown mean load tends to decrease.

The procedure can be very useful in the development of more realistic spacecraft sterilization specifications where the sterilization cycle is sensitive to the estimated number of buried contaminants. In particular, a significant decrease in the severity of the cycle may be possible if realistic estimates of the buried bioloads can be developed. (These benefits are not restricted to spacecraft applications but could also be extended into sterilization processes in the food and drug and other spin off areas.)

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[10] Exotech Incorporated, TRSR-036, "An Analytical Basis for Assaying Buried Biological Contamination", January 1969.

A preliminary analysis[11] of the probability of microbial release of buried contamination considered two release mechanisms, viz., break up in hard impact and aeolian erosion. Further, it formulated analytic expressions for the probability of release as a function of pertinent parameters and identified areas of uncertainty.

Specifically, the fracture ratio, i.e., the ratio of newly exposed area to the initial volume of material, upon which the non-nominal landing probability of release is especially sensitive, was one area of study. The results of impact tests[12] conducted by the Boeing Aircraft Company were reviewed and a method was devised for accounting for impact die off. The ratio of the viable spores recovered from the fragments to the initial load seeded into the pellet is a product of the release depth, the fracture ratio and the degree of impact die off. Values for release depth can confidently[13] be assigned in the 1 to 3 micron range. Fracture ratios can be determined by physical measurement of the recovered fragments. The accuracy of this measurement varies with the number and size of the fragments and has yet to be quantitatively bounded.

When values for release depth and fracture ratio are assigned, the recovered lethality ratio is then a direct measurement of the impact die off. This method of analysis will be applied when additional impact data becomes available.

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[11] Exotech Incorporated, TRSR 70-03, "Development and Application of a System Model for Spacecraft Sterilization", August 1969.

[12] S. J. Fraser, "Survival and Release of Viable Microorganisms After a Hard Impact", Boeing Company report #D2-114143-1, May 27, 1968

[13] N. J. Peterson, R. G. Cornell, and J. R. Puleo: "Release of Microbial Contamination from Fractured Solids." Paper presented at 11th Planetary Meeting of COSPAR, Tokyo, Japan, May 1968

Further study of theoretical fracturing may provide an insight into fracturing mechanisms. An upper bound to the area exposed during impact can be calculated from the kinetic energy at impact, knowing the energy required for a unit area of fracture. The materials of interest are mostly noncrystalline, buried viable spores are inconceivable in metal structures. Measurements in polymeric materials give 0.5 in.-lb/in^2 (about 10^5 erg/cm^2) for energy per unit area of fracture.[14] This estimate may be useful for preliminary upper-bound calculations of fracture associated with hard impact landings. Further work remains in this area.

Microbial release due to aeolian erosion was also studied and is reported in Appendix D[15]. Although a model has been developed, conclusions as to whether a release factor significantly less than unity for Mars can be invoked will require an agreed upon estimate of Martian meteorology. It is well known that astronomers have interpreted shifting haze that occasionally obscures the planet's features as being due to large sandstorms. Sand or dust is, of course, a major erosion agent, and simulations of the erosion effect of postulated sandstorms have been reported[16]. Analysis of the photography of the planet's surface by Mariner 6 and 7 tends to support the sandstorm hypothesis: the scarcity of small craters in the size distribution can be accounted for by an erosion process.

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[14] M. L. Williams, "The Fracture of Viscoelastic Material" in Fracture of Solids, D.C. Drucker and J. J. Gilman (ed,), Interscience (1963)

[15] M. J. Barrett and J. L. Woodall: The Release of Buried Microbial Contamination by Aeolian Erosion. Exotech Report No. TRSR 70-14.

[16] G. Dyhouse, "Simulated Martian Sand and Dust Storms and Effects on Spacecraft Coatings", ASTM/IES/AIAA Second Space Simulation Conference (Sept. 1967). Am. Soc Testing Materials, 1967, Philadelphia, Pennsylvania.

A second area of uncertainty that deserves investigation is the possibility of spore destruction during the erosion process The dimensions of a spore, typically on the order of a micron, are considerably smaller than those of the average sand particle encountered on Earth. Those dust particles in the micron range are generally more dominant in the stratosphere, and particle size can be loosely correlated with altitude since smaller size permits particles to be carried higher and farther by the wind. Presumably, the same mechanisms apply on Mars, and a lander in a Martian dust storm would be pelted by particles considerably larger than a spore. The erosion model to be applied, therefore, is akin to the use of boulders in chipping away seeds. One suspects a significant amount of lethality in this process.

Further study should concentrate on areas most conducive to permitting the unit value of probability of release to be reduced thereby alleviating sterilization cycle requirements. Analysis should be conducted to identify the areas of uncertainty where additional knowledge will produce the maximum benefit. Each factor should then be reviewed to permit selection of those most achievable within time and resource constraints. Effort should be expended only so long as a payoff in terms of further relaxation of the probability of release will result.

C. CHEMICAL CONTAMINATION

A common prerequisite for space exploration is the absence of contamination by terrestrial material in the area being explored. In the search for biological life, this concern has led to the establishment of rigorous planetary quarantine standards. The danger, however, exists in all science fields where experiment objectives can be compromised by the undesired introduction of unknown quantities of "foreign" matter.

In previous work[17], Exotech studied the problems of unintentional contamination of lunar samples to be returned to Earth for analysis.

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[17] Exotech Incorporated, TRSR 68-029, "Planning Study for an Organic Constituents Inventory Program", May 1968.

Adopting a philosophy that absolute purity cannot be guaranteed and that some contamination will be introduced by astronauts, vehicle ejecta and the capsule environment, an approach was developed which attempts to identify and quantify the types and levels of expected contamination. This is achieved by establishing an inventory of organic materials contained in Lunar landing spacecraft as part of an information system which can identify regions of high contamination risk and the probable contaminating materials.

The study undertaken in this contract defines the initial step in the implementation of the above approach. It considers the detailed procedures and tasks necessary to collect, evaluate, store and disseminate data which will serve anticipated needs of lunar sample investigators, consistent with the requirement that costs associated with implementation and operation of the inventory be compatible with known needs for this information. The primary tasks pertinent to this effort involved (1) determining the availability of lunar mission vehicle documentation and the means for collecting it in a form suitable for future evaluation, (2) the collection and utilization of spacecraft trajectory parameters, landing sites, and dispersion patterns for crash and soft landings, and (3) evaluation of the compatibility of required data inputs with an existing Planetary Quarantine information system. The final report[18] of this effort is appended.

Should further implementation be warranted, future efforts should include the following:

> Retention of documentation identifying types and quantities of chemical materials in lunar mission spacecraft landers.

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[18] Exotech Incorporated, TRSR 70-07, "Implementation of a Chemical Contaminant Inventory for Lunar Missions," December 1969

- Designation of a Federal Records Center to receive these documents and organization of a suitable filing system
- Preparation of a contamination risk model of the lunar surface based on the best available estimates of spacecraft landing sites and the particle dispersion associated with the mass and velocity characteristics of hard and soft landers.
- Dissemination among lunar sample investigators, and others associated with the planning of lunar scientific exploration, of information concerning the documentation to be kept in storage.

With the advent of landing missions to the planets of the solar system, similar attention is warranted to the question of chemical contamination by terrestrial material. The study should be extended to consider the acquisition of documentation and material samples from planetary flight missions. Information requirements of current and potential experimenting scientists who will analyze planetary materials should be collected. Information system designs which can serve these needs for data on material contamination should be formulated.

III. CONCLUSIONS AND RECOMMENDATIONS

1. Planetary Quarantine requirements, policies and constraints should be reviewed in the light of past experience and new knowledge to insure that maximum possible relaxation of sterilization specifications is realized.

   The implication on planetary quarantine requirements for approved flight projects of recommendations made by the Space Science Board at its December 11, 1969 meeting should be evaluated.

2. Flight project quarantine plans should be evaluated for their responsiveness to NASA PQ requirements and compatibility with accepted practices for their implementation.

3. Past planetary flights should be evaluated to develop realistic levels for the probability of contamination for future missions.

4. The probability that microorganisms contained on landing spacecraft will be released in a viable state on a planetary surface should be further studied. The effect of fracture upon impact and aeolian erosion during the period of biological exploration and the survivability of microorganisms in the course of the above release mechanisms should be considered.

5. Analytical models should be developed to facilitate the implementation of planetary quarantine requirements on flight projects through the utilization of new laboratory data or related technological progress in the following areas:

   (a) Estimation of microbial contamination in spacecraft environments.

   (b) Dry heat resistance of microorganisms on open surfaces, mated surfaces and buried contamination in spacecraft equipment. Microbial die off in dry heat sterilization should be further analyzed with emphasis on the role of moisture content.

(c) Thermal dynamics in heat sterilization of spacecraft equipment.

(d) System tradeoff model encompassing all relevant factors prior to, during, and after the application of heat sterilization.

6. The requirements for a planetary chemical inventory should be studied. Potential information requirements of scientists who will perform analyses of planetary materials to be collected during planetary flight missions should be identified, other material information systems should be investigated for their applicability.

7. A quarantine document system should be designed specifically oriented to serve the management of planetary quarantine on flight missions.

APPENDIX A

Potential Effects of Recent Findings on Spacecraft Sterilization Requirements

POTENTIAL EFFECTS OF RECENT FINDINGS ON SPACECRAFT STERILIZATION REQUIREMENTS

S. Schalkowsky[2]*, L. B. Hall[1] and R. C. Kline[2]*

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 has become available during the past year or which is 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.

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*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.

(1) National Aeronautics and Space Administration, Washington, D C.
(2) Exotech Incorporated, Washington, D. C

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 United States. 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.

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) and NASA (1966),'

$$P_c = N\,P(N) + N'\,P(N') \qquad (1)$$

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 microorganisms 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 ith 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

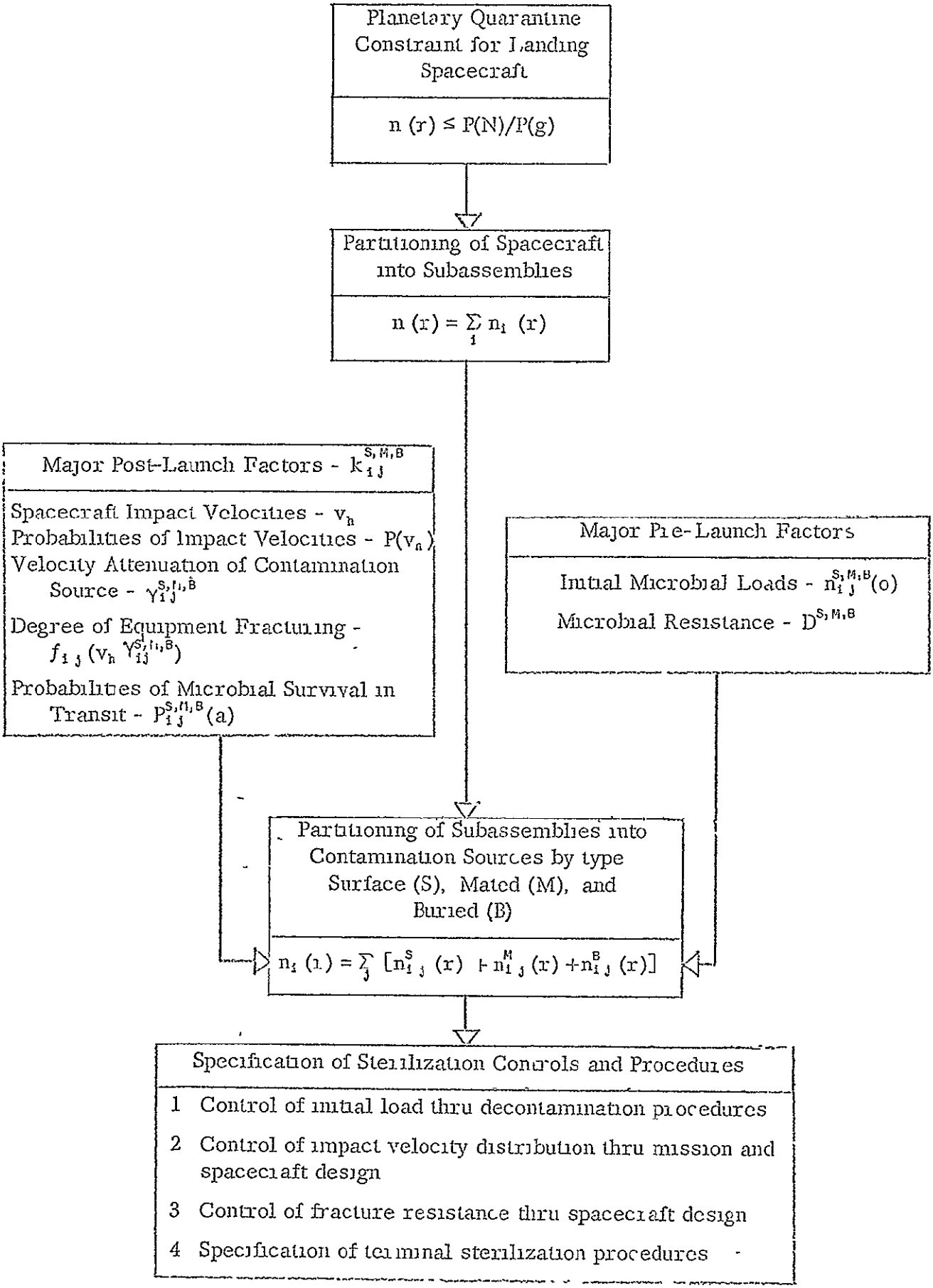

Figure 1 Major Considerations in the Specification of Sterilization Requirements

two release factors are intimately related to this partitioning. Thus, in order to evaluate microbial release caused by a crash landing from a particular source, 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 discussed 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 - $n_{ij}^{S,M,B}(o)$

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 - $D^{S,H,B}$

The resistance of microorganisms 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 microorganisms to heat sterilization. (Pflug, 1967 and 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 - $\gamma_{ij}^{S,fl,B}$

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 - $f_{ij}(v_h \cdot \gamma_{i\,j}^{B})$

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 noted herein by 'Peterson et al. (1968)' 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 microorganism 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 3 microns. 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 microorganism, i. e. about 1 μ. Considering the uncertainty in other parameters, it is of little consequence at present whether λ is taken to be 1 or 3 microns.

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. *

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* 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 260,000 pieces when the fracture ratio is about 1,200 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 microorganisms 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.

$$P(N)/P(g) \geq n(r) =$$

$$= \sum_i \sum_j \left[n_{ij}^S(o) \cdot P^S(s) \cdot k_{ij}^S + n_{ij}^M(o) \cdot P^M(s) \cdot k_{ij}^M + n_{ij}^B(o)\, P^B(s) \cdot k_{ij}^B \right] \quad (2)$$

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.

$$k^S_{ij} = P^S_{ij}(a) \quad P^S_{ij}(r) \qquad P^S_{ij}(1) = \begin{cases} 1, & \text{for exterior surfaces} \\ P(v_h), & \text{for interior surfaces if } v_h \geq v^S_{ij}/\gamma^S_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{3}$$

$$k^M_{ij} = P^M_{ij}(a) \quad P^1_{ij}(r) \qquad P^M_{ij}(r) = \begin{cases} P(v_h), & \text{if } v_h \geq v^1_{ij}/\gamma^M_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{4}$$

$$k^B_{ij} = P^3_{ij}(a) \cdot P^B_{ij}(r) \qquad P^B_{ij}(r) = \begin{cases} \lambda f_{ij}(v_h \quad \gamma^B_{ij})\ P(v_h), & \text{if } v_h \geq v^B_{ij}/\gamma^B_{ij} \\ 0, & \text{otherwise} \end{cases} \tag{5}$$

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

The velocities, $v^{S,M,B}_{ij}$, 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 microorganism 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.

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

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 $^{S\,x}$, 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

$$n(r) \leq n^{S\,x'}(o) \cdot P^{S\gamma}(a)\ 10^{-t/D^{S}} +$$

$$+ P(v_h)\left[n^{S}(o) \cdot 10^{-t/D^{S}} + n^{M}(o) \cdot 10^{-t/D^{M}} + \lambda \cdot 10^{-t/D^{B}} \sum_{i} \sum_{j} n^{B}_{ij}(o)\, f(v_h \cdot \gamma^{B}_{ij}) \right] \tag{7}$$

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 corollory 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 procedures. 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 parenthesis of equation 7, it is likely that work now in progress will show the product $\lambda \sum_{1} \sum_{j} n^{B}_{ij}(o) f(v_h \cdot \gamma^{B}_{ij})$ to be smaller than $n^{M}(o)$. 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, and, (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 microorganism spreading and growing on the planet surface.

REFERENCES

Committee on Space Research, Paris. COSPAR Resolution 26.5 COSPAR Information Bull , No. 33. Oct. 1966

National Aeronautics and Space Admin A Note on COSPAR Resolution 26.5. NASA Position Paper, May 1966.

Angelotti, R. Protective Mechanisms Affecting Dry Heat Sterilization. COSPAR Symposium on Sterilization Techniques, London, England. July 1967.

Pflug, I.J Dry Heat Sterilization Rates of Destruction and Temperature Coefficients. COSPAR Symposium on Sterilization Techniques, London, England. July 1967.

Peterson, N.J., R.G. Cornell, and J R. Puleo. Release of Microbial Contamination from Fractured Solids. Presented at the 11th Plenary Meeting of COSPAR, Tokyo, Japan, May 9-21, 1968. [Submitted to Space Life Sciences for publication]

Stern, J.A. (California Inst. of Tech., Jet Propulsion Lab.) Personal Communication. April 1968.

APPENDIX B

Investigations into a Diffusion Model of Dry Heat Sterilization

INVESTIGATIONS INTO A DIFFUSION MODEL OF DRY HEAT STERILIZATION

Interim Report

Contract NASw-1734
for
National Aeronautics and Space Administration
Office of Biosciences

Prepared by

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May 5, 1969

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TRSR-041

ABSTRACT

The analytical model described in this study formalizes the hypothesis that dry heat inactivation of microorganisms is closely related to the water content of the spore and its micro-environment. Experimental data are examined relative to this model and it appears to be valid. This model is aimed at overcoming the well known deficiencies of the logarithm model.

INTRODUCTION

Several recent investigations have related environmental conditions other than temperature to microbial spore survival under heat sterilization. Murrell and Scott, Angelotti, and Reid have shown strong dependency of dry heat death rates on ambient relative humidity and on water content of the spore*. Hunnell and Ordall have found that heat causes spores to exude calcium and dipicolinic acid (DPA) prior to death, while Alderton, Thompson, and Snell show that spores in aqueous suspension have improved heat resistance when calcium ions are present.

In addition to these correlations with environmental conditions during sterilization, spore survival shows correlations with environmental conditions that prevailed when the spore formed. Vinter shows heat resistance is affected by calcium and cystine availability during sporulation; Murrell and Warth show correlations of heat resistance with five different substances found in the spore.

These investigations show the importance of environment on heat sterilization characteristics. A simple chemical reaction does not appear to be the complete mechanism for spore destruction. Rather, a sequence of transport of chemicals, notably water, occurs and modulates the rate at which chemical reactions destroy the viability of the spore.

In this report we present a diffusion-denaturation model of spore heat resistance that attempts to correlate with the water effects observed, and to provide a basis for determining the efficiency of proposed dry-heat sterilization plans. Such a model is immediately useful in a dry-heat sterilization program, and also offers promise of eventual understanding of the remarkable resistance of spores to heat.

*References are listed at the end of this report.

DIFFUSION MODEL

A model to predict the water content of a spore as a function of time, temperature and initial water concentration within the spore has been developed. The assumptions made are that heat deactivation in spores is due to protein denaturation and that the rate of this reaction is controlled by the water content of the spore core.

A spore is composed of an outer coat (cortex plus spore coats) and a central core (cytoplasm) which is the dormant micro-organism. The cortex protects the core from physical damage, chemical contamination and rapid wetting by the environment. The cortex is mainly composed of mucopeptide polymers (Warth et al, 1963) with the chains twisted and coiled or interwoven (Mayall and Robinow 1957). The coat is mainly protein with a high cystine disulphide bond content (Vinter 1961).

The heat resistance of spores is assumed to rest in the ability to control the amount of water internal to the spore. A contractile cortex system (Lewis, Snell and Burr 1960) would provide a mechanism to dehydrate the cytoplasm and maintain it in this state. Chemical variation in the mucopeptide of the cortex and in the amount of Cu ++ or Ca-DPA (Young 1959) binding to the mucopeptide which may cause the contraction, could result in differences in the degree of contraction and therefore in the final water content of the spore, resulting in marked difference in heat resistance.

In the development of this diffusion model we have assumed a simplified spore structure composed of an outer coat of negligible thickness at a radius surrounding a spherical, homogeneous one.

Water transport thru the spore can be described by the well known diffusion equation

$$D\nabla^2 C = \frac{dC}{dt} \qquad (1)$$

where C is the concentration of water/cm^3, as a function of position and time. D is a diffusion coefficient depending on the medium.

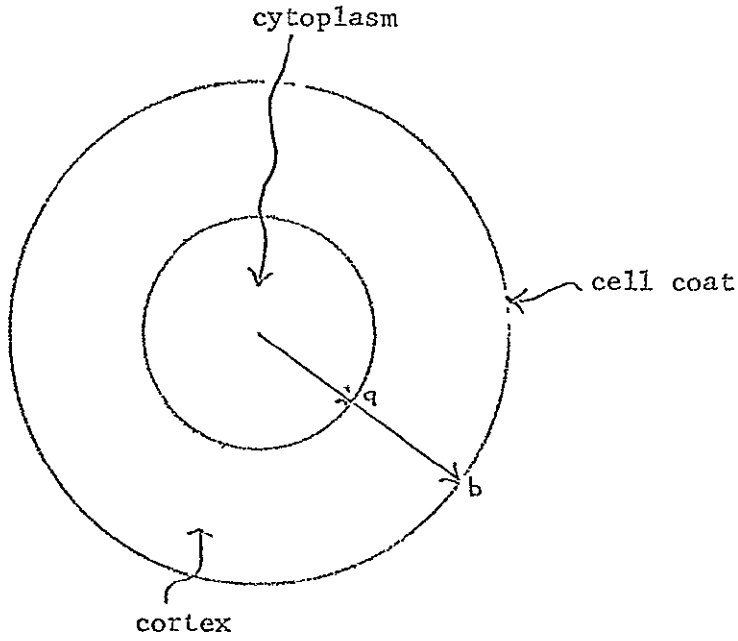

Figure 1. Model of Spore

For a simple example, assume the initial water content in a spherical spore is distributed radially according to

$$rC(r) = \frac{b}{\pi}\left[\sin\frac{\pi r}{b} - \tfrac{1}{2}\sin\frac{2\pi r}{b}\right] \qquad (2)$$

where C(r) is the water concentration at a distance r from the center of the spore, and b is the radius of the spore (see Fig 1). This expression, as seen in Figure 2, corresponds to a distribution that is peaked in the outer portion of the spore, and can be considered as approximating the case where a spore contains more water than an optimum amount. This excess water is stored in the cortex.

Applying the diffusion equation to this initial distribution results in the time variation shown in Figure 3. Water diffuses inward to the cytoplasm, and the spore gradually loses water to the medium. As a result, the initially-dry central region reaches a peak concentration of water that occurs around a time t given by

$$\frac{D\pi^2 t}{b^2} = 1.5 \qquad (3)$$

where D is the diffusion coefficient of the system. This simple model assumes D to be independent of position. As a result, it approximates the situation when the spore is buried in an appropriate medium, since normally one would expect the diffusion coefficient of water outside a spore to differ from D inside the spore.

Data have been reported by Angelotti (1966) for the thermal sterilization of spores in lucite. These data (Fig 4) indicate the death rate to be greatest at about 1.5 hours after commencing to heat the spores at 125°C. Using this value for t, in the above equation, we arrive at an estimate for the diffusion coefficient at 125°C

$$D = 1.2.10^{-6} \ (cm^2/sec) \qquad (4)$$

This estimate is in reasonable agreement with values for diffusion of protein molecules in water (Clark, p. 138). Our example requires the opposite water molecule diffusion in protein, which has an unknown diffusion coefficient,

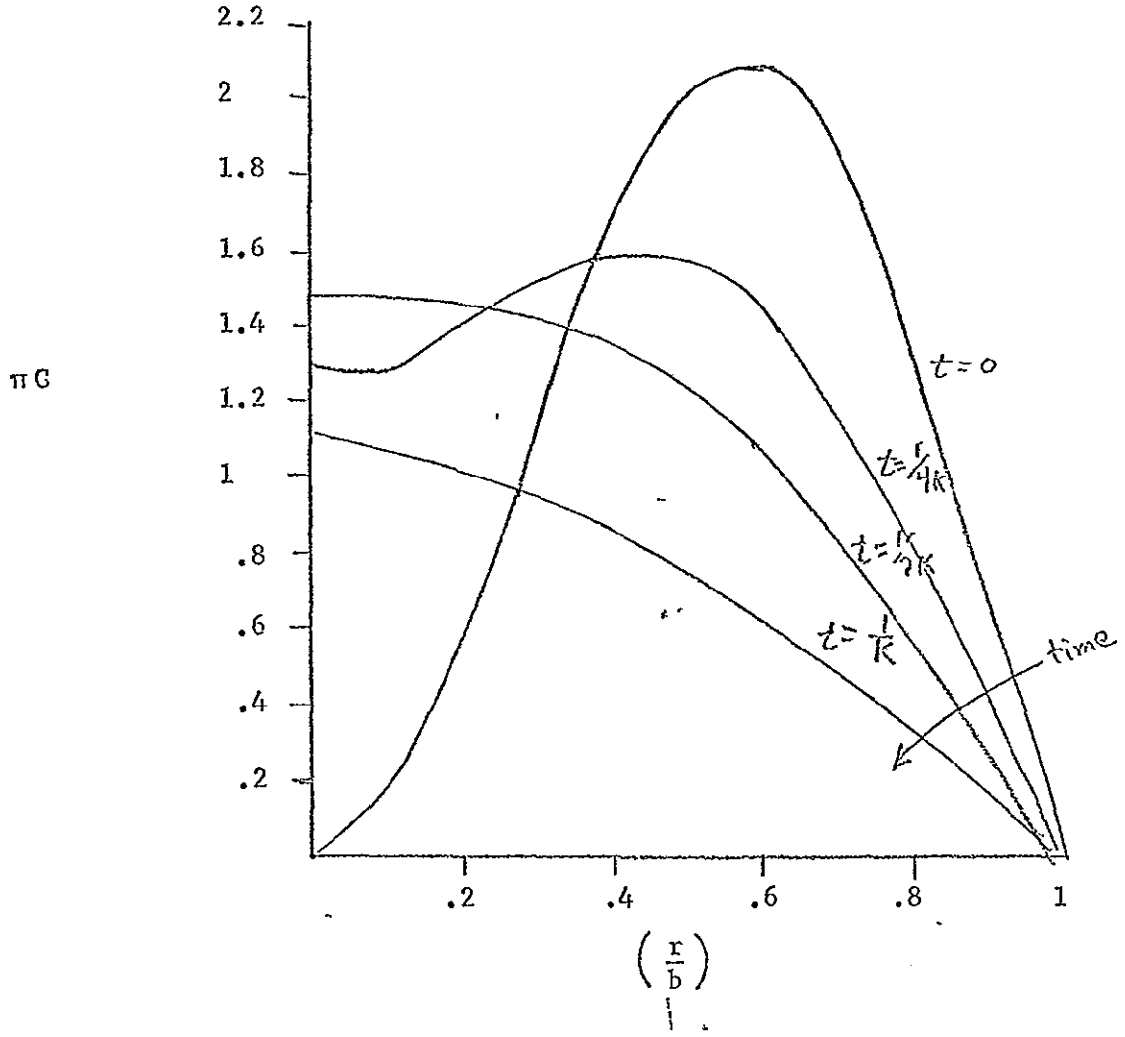

Figure 2. Water Distribution in the Spore

For $t = o$, $\quad rC = \frac{b}{\pi}\left[\sin\frac{\pi r}{b} - \frac{1}{2}\sin\frac{2\pi r}{b}\right]$

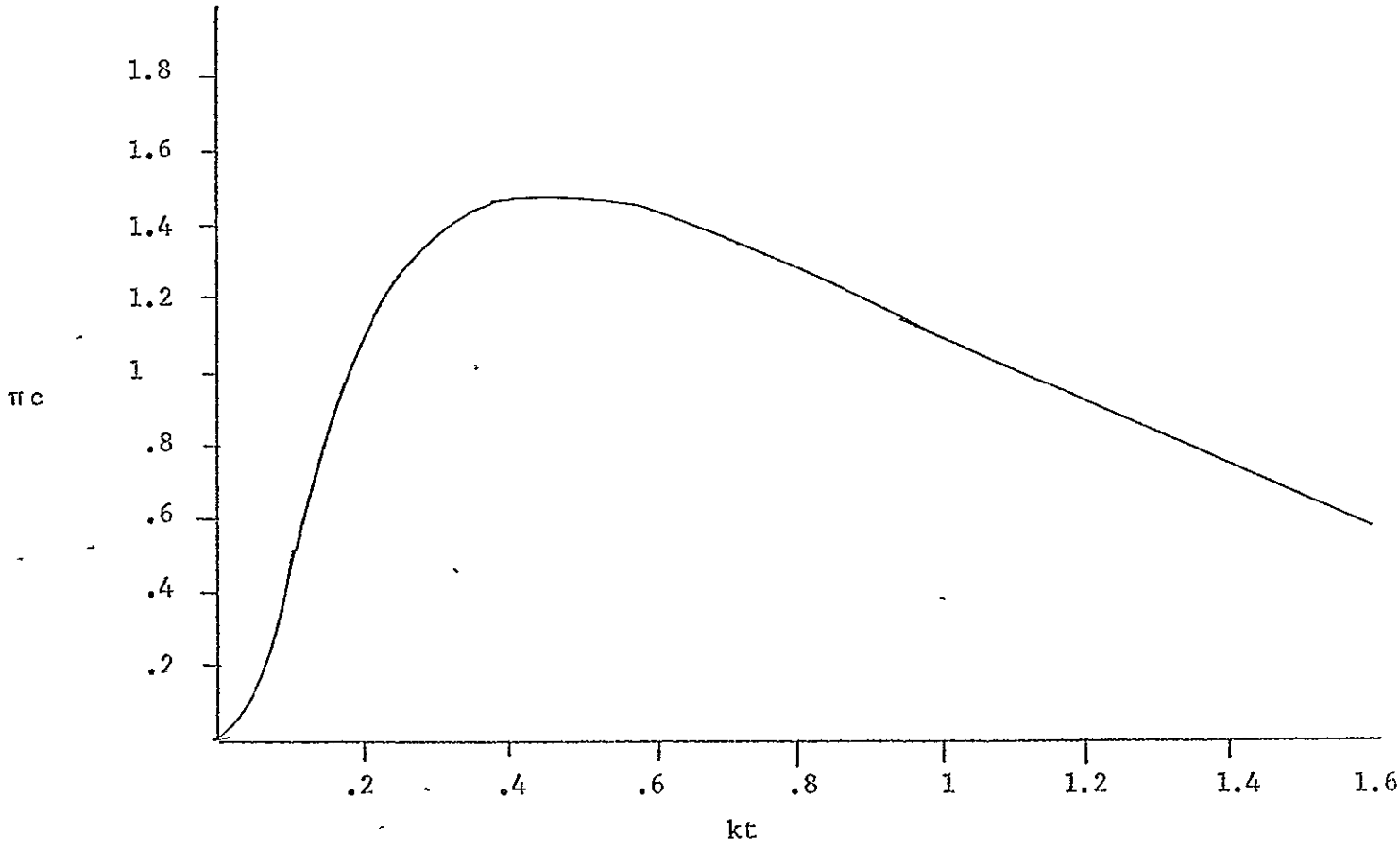

Figure 3. Concentration of Water at center of spore, as a function of heating time.

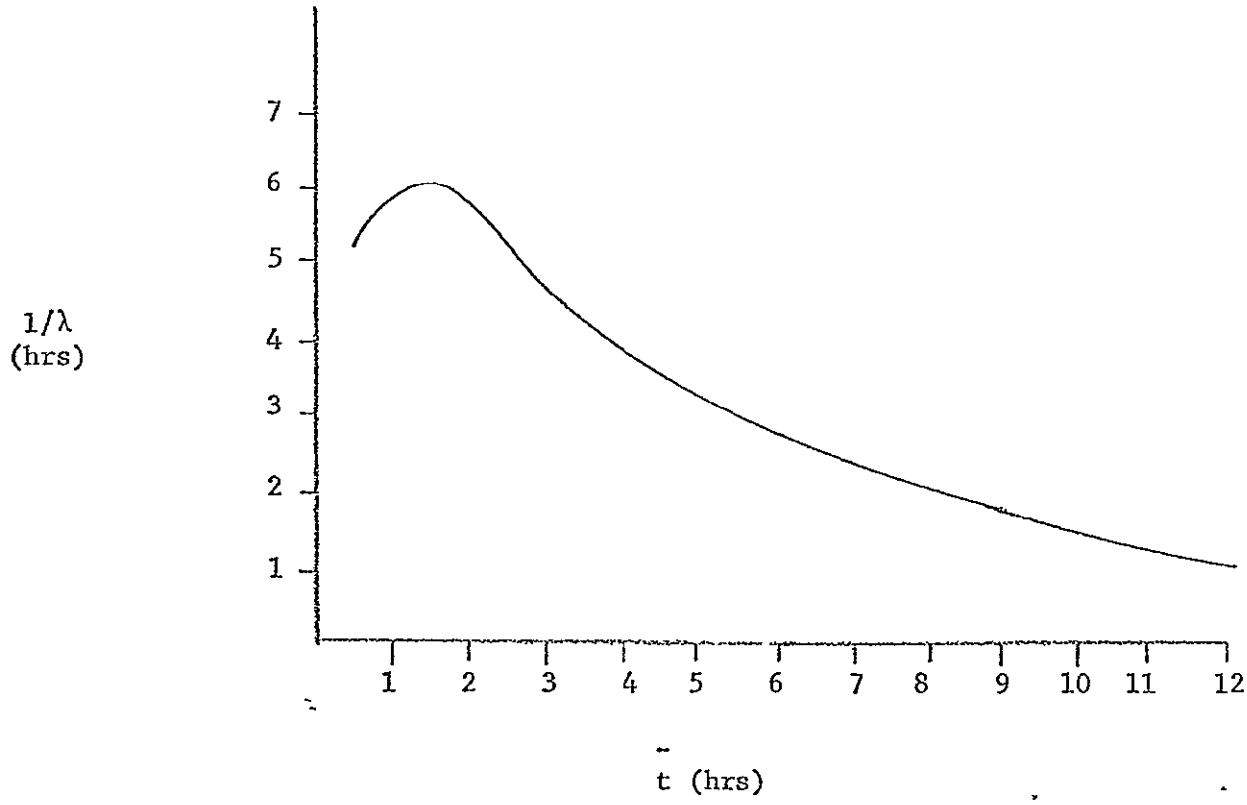

Figure 4. Apparent change in resistance of B. globigii in lucite after times $\bar{t}$ in hours at 125° C

and further assumes this coefficient does not differ greatly from water diffusion in lucite.

One method of evaluating the diffusion coefficient, which is necessary if a diffusion model is to be applied to dry sterilization, is to look at wet sterilization curves. Such curves are shown in Figure 5 for four temperatures. In wet heat, the spores should absorb water to some maximum. Then, the denaturation of proteins in the spore, with this water present, results in a straight logarithmic curve for survivors, as a function of time of heating.

Such a description fits the curves shown. The knee of each curve represents the time at which the cytoplasms of the spores are in equilibrium with the external water. For a purely exponential buildup of water, this occurs about when three relaxation times have passed, according to diffusion kinetics. The diffusion coefficient calculated by this method can be fitted to a formula

$$D = D_o e^{H/RT}, \text{ where } \begin{cases} H = 60 \text{ K cal} \\ R = 1.98 \\ T = \text{temperature } (^oK) \\ D_o = \text{Constant} \end{cases} \qquad (5)$$

Results of the calculations are shown in Figure 6, together with the diffusion coefficient calculated as Eqn. 5. The two sets of experiments seem to be in fair agreement, and indicate initial success in applying a diffusion model to the data.

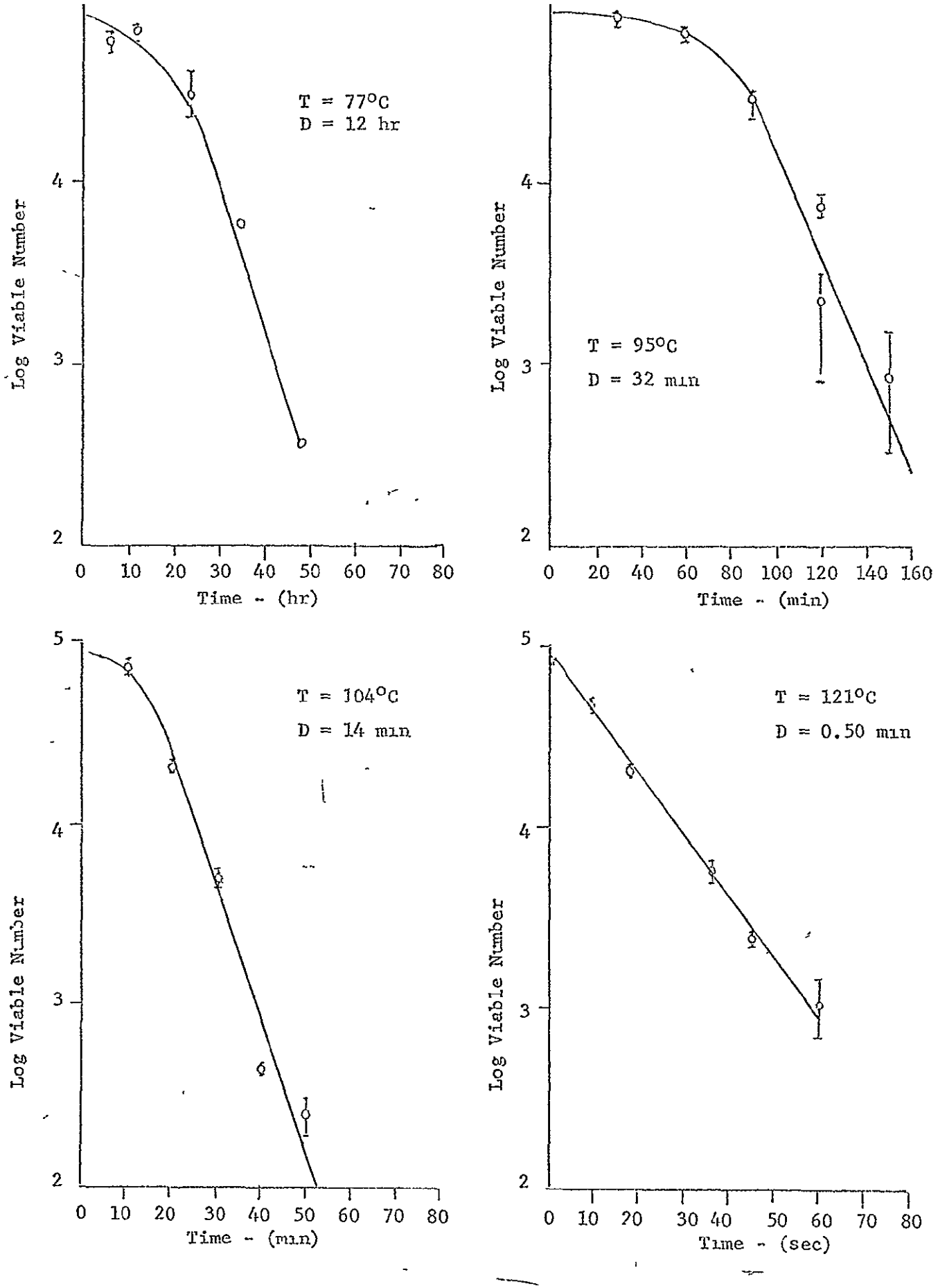

Figure 5. Wet Sterilization Curves (Data from Fox, Eder and Pflug 1967)

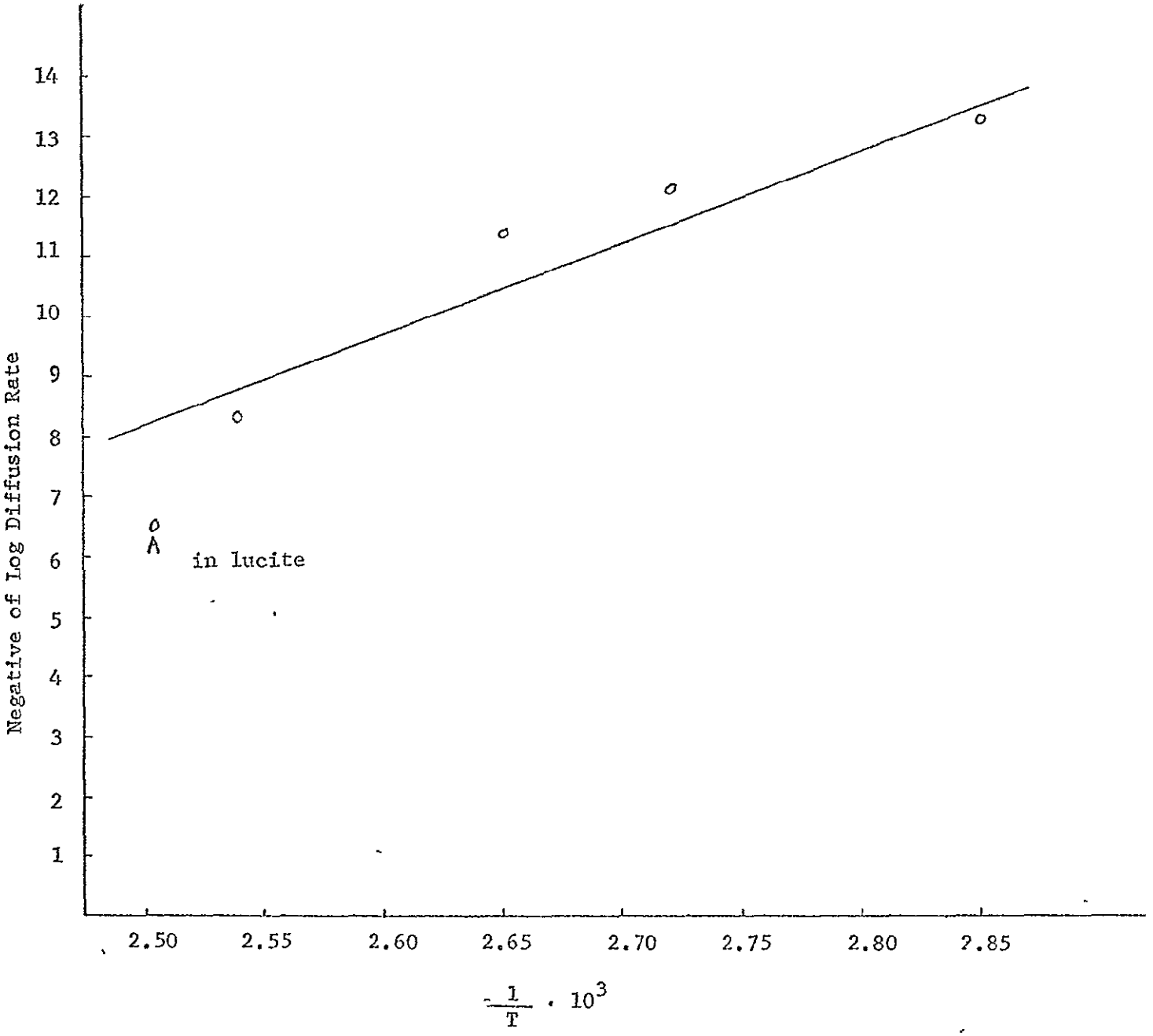

Figure 6. |log D| plotted versus 1/T
(Estimates based on wet heat data by Fox, Eder, Pflug)
(A· estimate from dry heat, by Angelotti)

IMPLICATIONS OF THE MODEL

The correlation of water activity with spore resistance indicates that the death mechanism is a denaturation of some vital protein. Much the same correlation has been observed between protein denaturation and water content. Furthermore, the reaction is generally of first-order kinetics, resulting in a logarithmic curve such as is frequently seen for thermal die-off of spore populations.

The vital protein is clearly in the cytoplasm, since the outer portions of the spore, shedded during germination, are not vital. This outer portion, the cortex, appears to squeeze water from the cytoplasm during the formation of the spore, and thereby provides that there will be a residual concentration that is near the optimum for heat resistance.

Part of the heat resistance of spores has been shown to be due to calcium dipicolinate, manufactured during the formation of the spore, and present in the cytoplasm. Ca-DPA is a chelating agent. Presumably, the vital protein has sensitive bonds that are protected by Ca-DPA and other sensitive bonds protected by water molecules.

Germination of the spore presumably requires that the protein be rid of these protections. Excess water can remove the Ca-DPA; the attached water molecules may separate thermally (heat shock). These separations, if reversible, would be of little help to germination unless the protecting molecules stripped from the protein were to leave the cytoplasm. This is the argument for diffusion: it allows proteins to react with enzymes, etc., in the germination process without hindrance from the former bond-protecting agents. At the same time, the proteins become more sensitive to heat.

Diffusion is the random movement of molecules. It is characterized by straight-line paths between molecule interactions, and arbitrary change of direction after the interaction. The net result of the random motion is a movement of molecules from regions of high density to regions of low density. Frequently, these interactions are mere collisions. In such a case, the diffusion constant D is proportional to

temperature. A less frequent situation is where the collisions involve chemical reactions. The molecule moves in a straight line, collides and "sticks" to a fixed obstacle, is freed by the action of heat, and moves off in an arbitrary direction. This kind of diffusion, characterized by a diffusion constant as given in Eq. 5, appears to fit the process of water diffusing through the spore cortex.

The environmental conditions prevailing during spore formation have been shown to affect the subsequent resistance of the spore to heat. This, too, is as the model would imply. There is no known mechanism by which the spore can control the water concentration in the cortex and spore coats. As a result, the moisture content of these regions will vary so that they are in equilibrium with external conditions. When the spore is heated, the contents of these regions can diffuse inward and outward to affect the heat sensitivity of the spore.

These two mechanisms - diffusion and chemical reactions of proteins - appear responsible for the survival probability of spores as a function of humidity, temperature and time. Much work remains in the analysis of their quantitative aspects. What are the equilibrium moisture contents of spores? What is the water distribution inside the spore? What are the surface transfer properties? Does the Ca-DPA diffuse, too? What is the protein denaturation reaction that occurs? How many protein molecules must denature before the spore becomes nonviable? The answers to these questions require further study. Many previous experiments, unfortunately, are of little help since not all the pertinent variables were measured.

RECOMMENDATIONS FOR FUTURE WORK

1. Moisture Content Analysis

Measurements of the moisture content of spores are needed. For ease in comparing results of different environments, only one species (B. subtilis var. niger is the accepted norm) should be used and standard harvesting, washing, and drying procedures employed. Selection of procedures is a subject for discussion, but standardization will permit studies of the results to be concentrated on the environmental effects.

The environments to which the spores are equilibrated can vary in temperature, in humidity, and in length of time of equilibration. Down-side and up-side equilibration need separate studies, in view of the "hysteresis" effect observed. The rate at which spores give off water during an analysis, together with the cumulative water emission, should be measured.

These measurements should be analyzed to see whether they conform to the hypotheses discussed in this report. Evaluations of diffusion coefficients and surface transfer effects should be possible.

2. Correlation of Moisture and Sterilizability

Dry heat sterilization, with moisture content as a parameter, has been measured with uncertain results. The understanding gained from the analysis above should provide benchmarks for future measurements of spore sterilization rates. The early work sometimes suffered from a lack of determination of the pressure or the relative humidity during sterilization. The substrate deserves attention: if it has good water transference properties it can affect the experiment significantly.

With knowledge of moisture transfer properties in the spore, on its surface, and through the environment, it should be possible to

design crucial experiments where the moisture content and temperature of the spores are known quantities. The die-off of spores under these conditions should be measured. Such experiments include:

(a) Spores in vacuum. The moisture transfer outside the spores is a relatively easy calculation.

(b) Spores in non-permeable materials (e.g. epoxy). With different initial moisture contents, spores hould show different die-offs, but each experiment should provide nearly logarithmic curves.

(c) Spores in air. Moisture transfer is affected by the relative humidity and this can be varied in a sequence of experiments.

(d) Spores of different initial A_w in air.

REFERENCES

1. Alderton, G.; Thompson, P. A.; and Snell, N.: "Heat Adaptation and Ion Exchange in Bacillus Megaterium Spores"(1964) Science 143, 141-143.

2. Angelotti, R.: "Ecology and Thermal Inactivation of Microbes in and on Interplanetary Space Vehicle Components" Fourth Quarterly Report of Progress on Research Project R-36-015-001 March 1966, 1967, 1968.

3. Black, S. H.; Hashimoto, T.; and Gerhardt, P.· "Calcium Reversal of the Heat Susceptibility and Dipicolinate Deficiency of Spores Formed Endotrophically in Water" (1960) Can. J. Microbiol 6 212-224.

4. Clark, W. M.· "Topics in Physical Chemistry" p. 138 (1948) The Williams and Wilkins Co., Baltimore.

5. Halvorson, H. and Howitt, C.: "The Role of DPA in Bacterial Spores" (1961) Spores II H. Halvorson (ed) Burges Publishing Co., Minneapolis.

6. Hunnell, J. W. and Ordall, Z. J.· "Cytological and Chemical Changes in Heat Killed and Germinated Bacterial Spores" (1961) Spores II H. Halvorson (ed) Burges Publishing Co., Minneapolis.

7. Lewis, J. C.; Snell, N. S.; and Burr, H. K.: "Water Permeability of Bacterial Spores and the Concept of a Contractile Cortex" (1960) Science 132: 544-545.

8. Maynall, B. H. and Robinow, C. F.. "Observations with the Electron Microscope on the Organization of the Cortex of Resting and Germinating Spores of Bacillus Megaterium" (1957) J. Appl.Bacteriol. 20 333-341.

9. Murrell, W. G. and Scott, W. J.: "The Heat Resistance of Bacterial Spores at Various Water Activities" J. Gen. Microbiol. (1966) 43, 411-425.

10. Slepecky, R. and Foster, J. W.· "Alteration in Metal Content of Spore of Bacillus Megaterium and the Effect on Some Spore Properties" (1959) J. Bacteriol 78 117-123.

11. Vinter, V.· "The Formation of Cystine-Rich Structure in Sporulating Cells and its Possible Role in the Resistance of Spores" (1961) Spores II H. Halvorson (ed) Burges Publishing Co., Minneapolis.

12. Warth, A. D.; Ohye, D. F.; and Murrell, W. G.· "The Composition and Structure of Bacterial Spores" (1963) J. Cell. Bio. 16 579-592.

13. Young, I. E.· "A Relationship Between the Free Amino Acid Pool, Dipicolinic Acid and Calcium from Resting Spores of Bacillus megaterium" (1959) Can. J. Microbiol. 5 197-202.

APPENDIX C

An Analytical Basis for Assaying Buried Biological Contamination

AN ANALYTICAL BASIS FOR ASSAYING BURIED BIOLOGICAL CONTAMINATION

Interim Report

Contract NASw-1734

for

National Aeronautics and Space Administration

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SECTION 1 - Introduction

SUMMARY AND EVALUATION

This document prescribes and evaluates a procedure for estimating upper bounds on the mean concentration of viable organisms buried within individual spacecraft materials.*

Presented herein is an analysis of a procedure for assaying biological contamination buried or embedded in spacecraft materials. The procedure requires the controlled fracture of representative samples of a material whose buried loading is of interest. Each sample is tested for biological contamination on the totality of surfaces exposed as a result of the fracturing process. The basic datum or observation consists of the proportion of samples which yield contamination upon culturing. Conventional statistical techniques, combined with an assumed relation between the mean concentration of organisms buried within the material and the observed datum, produce an upper bound estimate for the unknown mean concentration, expressed to any prescribed level of confidence. In principle, the "conservativeness" of the resulting estimate is directly related to the sample size and the amount of surface area exposed by fracture, as the sample size and/or exposed area increase(s) the difference between the estimate and the unknown mean load tends to decrease.

The procedure, if feasible in terms of accuracies derived, engineering practicality and economics, would be very useful in the specification of realistic spacecraft sterilization requirements. This follows from the fact that sterilization requirements are quite sensitive to the release of buried contamination. Significant decreases in these requirements may be possible if realistic estimates of the buried bio-loads are made available. Conventional bio-assay techniques are impractical for most applications to spacecraft materials since they require that the materials be either pulverized or dissolved. The procedure discussed herein requires neither of these actions and, moreover, requires no direct counting of viable organisms.

There are potential shortcomings in the proposed procedure. In particular, there may be practical engineering difficulties or cost considerations which limit the application of the technique. Moreover, the accuracies resulting from its application could turn out to be less than desired. For these reasons, it is important that tests and further analyses be conducted to resolve these questions before steps are taken to operationally implement the procedure.

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*The concept underlying the procedure discussed herein was originally suggested by L. Hall, Office of Biosciences, National Aeronautics and Space Administration.

SECTION 2 - Background

SENSITIVITY OF MISSION REQUIREMENTS TO THE RELEASE OF BURIED CONTAMINATION

Sterilization requirements for individual lander missions are quite sensitive to the release characteristics of buried contamination. Effective techniques for assaying buried contamination could lead to substantial decreases in these requirements.

Recent analyses indicate that spacecraft sterilization requirements for planetary lander missions are quite sensitive to the release characteristics of contamination buried (embedded) within spacecraft materials.[1] In fact, under the majority of presumably realistic situations studied, the threat of buried contamination was the controlling factor in the determination of sterilization requirements. Within the context of the subject analyses, this result was attributed to the relatively high resistance of buried contamination to sterilizing temperatures, as compared with resistances of contamination located on open and between mated surfaces (resistance being represented by the D-value parameter of the exponential survival curve).

The relatively high resistance of buried contamination presently assumed (viz. $D_{125°C}$ = 5 hours) was not the only factor contributing to the dominance of buried contamination in the determination of sterilization requirements. Two other major contributors were (1) the break-up characteristics of spacecraft materials containing buried contamination and (2) the amount of contamination actually buried within these materials, i.e. the threat of buried contamination is directly related to the existing amount of contamination and its accessibility to a planetary environment upon impact. The lack of definitive data relating to these factors presently necessitates a pessimistic view of their quantitative effects on sterilization requirements. Therefore, in exploring alternatives for decreasing sterilization requirements on individual lander missions, consideration should be given to justifying less pessimistic representations of the effects of these factors. This could be accomplished by determining more realistic estimates of spacecraft break-up characteristics and buried bio-loadings.

The break-up characteristics of spacecraft materials should be and are being investigated.[2] However, there are inherent difficulties associated with the quantification of this aspect of the buried biological threat. For example, there are practical problems associated with measuring the amount of break-up and relating it to flight path parameters such as impact velocity. In fact, very little is presently known about the fracturing characteristics of the many varieties of spacecraft materials. In many respects, determination of the amount of buried contamination is less complicated than the break-up problem. For example, the magnitude of the buried bio-load has nothing to do with the uncertainties of the mission flight path whereas the amount of spacecraft break-up is intimately related to the mission flight path parameters. The extensive background material in existence concerning biological loadings and their measurement, also suggests it to be a more fertile area of investigation.

SECTION 2 - Background

DRAWBACKS OF CONVENTIONAL BIO-ASSAY TECHNIQUES

Conventional techniques for assaying buried contamination are impractical for applications to most spacecraft materials since they require that materials be either pulverized or dissolved.

Standard laboratory procedures for the detection and enumeration of buried contamination fall into two major categories. One class of procedures requires that the subject material be broken into very small pieces (i.e. pulverized) and that direct counts of the exposed organisms be made. A second class of procedures requires that the material under investigation be dissolved in a suitable solvent which is non-toxic to the buried organisms. This second procedure also involves the subsequent counting of exposed organisms.

Several drawbacks are inherent in the application of the above techniques, especially where spacecraft materials are involved. Basically, there are serious questions relating to the practicality of pulverizing or dissolving most spacecraft materials (as opposed to laboratory application of those techniques which mainly involve materials that can be appropriately pulverized or dissolved). In the particular case of pulverization techniques, the basic objective is to reduce the solid to particles of a size which essentially releases all organisms that are present without damaging the individual cells. Significant numbers of organisms usually go undetected since the chance of releasing all of them is very small. In addition, it has been found that the pulverization process itself damages or "kills" significant numbers of organisms, thus rendering them undetectable. For the most part, these two engineering problems prohibit a precise assay, if the pulverization is complete enough then it is likely that a significant number of organisms will be damaged in the process. The analogous problem associated with the use of solvents is that very few types of materials can be dissolved without using combinations of heat, pressure and chemicals which destroy the buried organisms. Finally, both classes of procedures are appropriate only when counting high concentrations of contamination. They are ineffective or, at best, inefficient when applied to the low numbers of organisms buried within aerospace hardware. Their reliability for measuring low densities, (e.g. less than one organism per cubic centimeter) has not been adequately established.

SECTION 3 - Development of the Bio-Assay Procedure

BASIC CONCEPTS AND ASSUMPTIONS UNDERLYING THE PROPOSED PROCEDURE

The proposed procedure is premised upon a more-or-less uniform distribution of buried contamination and the release of the biological contents of a subvolume of material exposed through fracturing.

Suppose a given volume of homogeneous spacecraft material which contains buried contamination is fractured into several distinct pieces. It is reasonable to assume that the fracturing process effectively exposes the biological contents of a subvolume, V_e, of the interior of the material. Analysis of recent fracturing experiments[1] indicates that Expression (1) on the facing page is an acceptable representation of the exposed subvolume. In this expression A_e denotes the surface area newly exposed as a result of fracturing and λ denotes an effective depth of penetration, i.e., the depth beneath the exposed surface to which previously buried contamination is released. For convenience, λ is designated the "exposure depth coefficient". This concept of a subvolume exposed by fracturing was applied to the data obtained from the previously mentioned experiments. Estimates of λ evolved which ranged between one (1) and three (3) microns. This range has intuitive appeal in that it encompasses the mean diameter of microbial spores.

It is reasonable to assume that contamination buried within an homogeneous material is, for the most part, uniformly distributed throughout the interior. Moreover, assuming a standardization or uniformity of parts production procedures suggests that the concentration of contamination per unit volume of material is randomly distributed about some fixed value, C. These observations, along with recognition of an essentially unending source of contamination in production environments suggest the Poisson distribution as an appropriate formulation for describing the dispersion of buried contamination within homogeneous solid materials. This being the case, the probability that exactly K organisms are contained within the exposed subvolume V_e is given by Expression (2). In this expression N_e denotes the number of organisms exposed through the fracturing process.

The preceding two assumptions on the effective subvolume of material exposed by fracturing and the uniform dispersion of biological contamination within the selected materials constitute the basis for the analytical bioassay procedure presented and applied in the remainder of this report.

Effective Subvolume of Material Exposed Through Fracturing

$$V_e = \lambda A_e \tag{1}$$

λ - Exposure depth coefficient
A_e - Exposed surface area

Probability that Exactly K Organisms are Exposed Through Fracturing

$$P\{N_e = K\} = \frac{(\lambda A_e C)^K}{K!} e^{-\lambda A_e C} \tag{2}$$

$$K = 0, 1, 2 \cdots$$

C = Mean concentration of organisms per unit volume of material.

SECTION 3 - Development of the Bio-assay Procedure

THE BASIC MATHEMATICAL RELATIONSHIP (MODEL)

The probability that buried contamination will be exposed through fracturing is obtained from the assumed Poisson distribution. The resulting representation is the basic relationship or model underlying the proposed bio-assay procedure.

The probability that buried contamination will be exposed when a solid material is fractured can be obtained from the previously developed form of the Poisson distribution (Expression (2)). It coincides with the Poisson probability, p, that at least one viable organism is contained in the effective subvolume of material exposed as a result of the fracturing process. The expression for this probability, indicated by Expression (3) on the facing page, constitutes the basic relationship underlying the proposed bio-assay procedure. For convenience, it is rewritten in Expression (4) as a relationship which specifies the mean concentration, C, of organisms per unit volume of material in terms of the parameters λ , A_e, and p.

In principle, if values of λ , A_e, and p are specified, then the unknown concentration, C, can be determined on the basis of Expression (4). In practice, none of the above parameters can be determined or controlled exactly. The exposure depth coefficient, λ , can be estimated on the basis of experimental laboratory data on varied materials. (The previously mentioned estimates of λ were determined on the basis of Expression (3), controlled values of C and A_e and experimentally obtained estimates of p.) Assuming that the area, A_e, exposed through fracturing can be controlled sufficiently, estimates of p can be obtained experimentally. These estimates of p can be converted to upper bound estimates of C to any level of confidence on the basis of Expression (4) and standard statistical estimation techniques.

Probability that Buried Contamination is Exposed Through Fracturing

$$
\begin{aligned}
p &= P\left\{ N_e \geq 1 \right\} \\
&= 1 - P\left\{ N_e = 0 \right\} \\
&= 1 - e^{-\lambda A_e C}
\end{aligned}
\tag{3}
$$

The Mean Concentration of Buried Contamination Per Unit Volume of Material

$$
C = \frac{-\ell n\,(1 - p)}{\lambda A_e} \tag{4}
$$

SECTION 3 - Development of the Bio-assay Procedure

STATISTICAL LOAD ESTIMATION PROCESS

An upper bound estimate of the mean bio-load, expressible to any level of confidence, is determined from Expression (5) and experimentally obtained estimates of the probability that buried contamination will be exposed by fracturing.

Suppose that s samples of a specific spacecraft material are selected at random (e.g., the samples could be piece parts constructed from a particular homogeneous material). Further, suppose that each sample is fractured exposing a predetermined amount of new surface area, the same area being generated for all samples. Each sample is classified as "positive" if and only if biological contamination is found on the newly exposed surface area upon culturing. Assuming all samples are processed according to a fixed experimental procedure, the number of positives is Binomially distributed with "success" parameter p, as defined in Expression (3). Hence, the proportion, $\hat{p}$, of positives has an average or mean value p and is distributed as specified in Expression (5) on the facing page. Knowledge of the distribution of $\hat{p}$ allows for the determination of arbitrary confidence intervals for the unknown p, expressed in terms of observed values of $\hat{p}$. Appendix A contains a procedure for obtaining confidence intervals for p in terms of $\hat{p}$ and the sample size s. Figures I - B through IV - B of Appendix B are displays of 50, 80, 90 and 95 percent confidence intervals for p, respectively, determined using the procedures outlined in Appendix A. The various curves in each figure correspond to selected sample sizes.

Since the mean bio-concentration, C, specified in Expression (4) is an increasing function of p, upper confidence limits for p can be transformed, via this expression into corresponding confidence limits on C. Since this transformation involves the exposed area as well the sample size, a given confidence limit on p transforms into a distinct confidence limit on C for each value of exposed area. Figure V - B through VIII - B of Appendix B display the resulting upper confidence limits on C, corresponding to selected values of s, A_e and the observed proportion, $\hat{p}$, of positives. It should be noted that the lower confidence limits on p are not transformed into limits on C in these figures since primary concern here is with upper bound limits.

Figures V - B through VIII - B of Appendix B provide the necessary tools for testing and implementing the proposed analytical bio-assay procedure. The remainder of this report contains a step-by-step procedure for applying these figures, an illustrative application and a discussion of potential sources of load estimation error associated with the procedure.

$$p\left\{\hat{p} = \frac{k}{s}\right\} = \binom{s}{k} p^{k}(1-p)^{s-k} \qquad (5)$$

$k = 0, 1, 2, \text{---}, s$

SECTION 4 - Applications of the Bio-assay Procedure

SUMMARY PROCEDURE AND ILLUSTRATION

The preceding development is converted to an operational procedure using Figures V-B thru VIII-B of Appendix B.

On the basis of the preceding development, a protocol for establishing upper bound estimates of the mean bio-load buried within a given spacecraft material is summarized as follows:

1. Determine an appropriate combination of sample size, s, and area to be exposed, A_e (this decision is based, for the most part, upon "fracturability" and cost considerations)

2. Select at random a number, s, of samples of the given spacecraft material.

3. Fracture each sample so as to yield the selected amount, A_e, of newly exposed area.

4. For each sample, establish whether viable organisms were exposed upon fracture. Let $\hat{p}$ denote the proportion of samples which yield contamination, i.e. $\hat{p}$ is the proportion of positive samples.

5. On the basis of s, A_e and $\hat{p}$ and the desired level of confidence read the corresponding upper bound estimate of the mean bio-load on the appropriate graph from Figures V-B through VIII-B of Appendix B.

The indicated graphs do not allow for arbitrary selections of the sample size and exposed area. In the event that curves corresponding to other values of these parameters are needed, they can be determined from Expression (4) and Figures I-B through IV-B of Appendix B.

To illustrate the procedure, suppose that 25 samples of a given spacecraft material are selected and each sample is fractured, yielding an exposed area of 2.5 square inches. Suppose that 15 of these samples display contamination on the newly exposed surfaces. Finally, assume that an upper bound estimate of the mean bio-concentration is desired at the 90% confidence level. It is determined from Figure VIII-B of Appendix B, i.e. the graphical display corresponding to the 90% confidence level. The appropriate curve in this figure is the one corresponding to the given sample size, i.e. s=25. The observed proportion of positive samples is given by $\hat{p} = 15/25 = 0.6$. This value on the horizontal axis of the figure determines the appropriate point on the s=25 curve. Using the vertical scale corresponding to the

exposed area $A_e = 2.5$ in^2, an upper bound estimate of the mean bio-concentration is seen to be less than 1.04×10^4 viable organisms per cubic inch with 90% confidence. This concentration, when multiplied by the total volume of subject material on the spacecraft, produces an upper bound estimate of the bio-load buried within the given material.

SECTION 5 - A Preliminary Evaluation of the Bio-assay Procedure

SENSITIVITY OF LOAD ESTIMATES TO THE CONTROL AND MEASUREMENT OF λ AND A_e

To a first order approximation, percent errors in the measurement of either λ or A_e induce equivalent percent errors in estimates of the mean concentration of buried contamination.

As indicated in Expression (4), the mean concentration of buried contamination is inversely proportional both to the exposure depth coefficient, λ, and to the exposed area, A_e. Therefore, to a first order approximation, percent errors in the measurement of either one of these parameters result in identical percent errors in the mean concentration of buried contamination. Although this statement cannot be extended beyond certain limits, it does provide an approximate quantitative measure of the effects of measurement errors in λ and A_e on estimates of the unknown mean concentration, C.

As noted earlier, experimentally determined estimates of λ ranged between one and three microns for a specific material (lucite) and particular measurement procedures. If the "true" value of λ lies within this range for all spacecraft materials then a maximum of 300% error in λ is possible (i.e., assuming λ equals 3 microns but is estimated to be 1 micron). Assuming that order or magnitude estimates of spacecraft bio-loads are sufficient for most applications, errors of the above magnitude appear to be acceptable. In any case, taking λ as the lower limit of the range of estimates (i.e., 1 micron) provides more concervative upper bound estimates of C than would any other value selected in the given range, this is consistent with sterility assurance. There is no question, however, that estimates of λ corresponding to materials other than lucite is desirable, if not mandatory.

There is a sparsity of both theoretical and empirical data on the control and measurement of surface areas exposed by fracturing materials of the types used in spacecraft construction. Moreover, the implications of a given distribution of measurement errors in A_e are, at best, difficult to derive on a statistical basis owing to the relatively complex relationship between A_e, the observed datum, $\hat{p}$, and the estimated mean biological concentration. For these reasons, it is difficult to speculate on the errors introduced into bio-assay estimates as a result of incorrect measurements of exposed areas. The previously referenced Pheonix experiments [1] failed to provide sufficient data for resolving these questions completely, even as applied to lucite. However, evaluation of the experimental procedures and the resulting data does suggest that, for this particular material, the area control and measurement procedures used along with selecting λ equal to one micron is adequate for present purposes. Here again, extrapolation to other materials may not be valid, hence, additional data in this regard is warranted.

SECTION 5 - A Preliminary Evaluation of the Bio-Assay Procedure

ADDITIONAL SOURCES OF POTENTIAL ERROR

Potential sources of error related to deficiencies in the sampling and culturing processes as well as the analytical model itself indicate the need for controlled tests of the bio-assay procedure.

The proposed procedure evolved, in part, from the assumption of a Poisson distribution of viable organisms within the interiors of materials being assayed. Although this representation has intuitive appeal for most applications, a test and validation phase is nevertheless necessary before implementation is considered.

Estimation errors are likely to occur if improper sampling, and/or culturing procedures are followed. In sampling material, care must be taken to insure that a representative cross-section is selected, i.e., samples independently taken from distinct batches of the given material. Otherwise, the selected sample size could be insufficient for attaining a desired confidence level. The culturing procedure is intimately connected with the exposure depth coefficient, λ , since the depth of penetration is likely to vary with the nature or type of culture medium. For any given depth of penetration, however, the culturing process should be capable of detecting all viable organisms which are exposed.

It is important to note that the proposed bio-assay procedure, if successful, produces upper bound estimates of the mean concentration taken over the total population of the sample material under investigation. This is less than desirable from the standpoint of application to sterilization requirements for individual lander missions. For example, it is possible, though quite unlikely, that the dispersion of concentration from sample to sample is very large. If so, an upper bound estimate of the mean concentration to any level of confidence could have a relatively high probability of being less than the concentration of a randomly selected sample and spacecraft. Further testing and analysis are warranted on this basis alone.

SECTION 6 - Recommendations

THE NEED FOR TESTING AND FURTHER ANALYSIS

Tests and additional analysis of the usefulness, engineering practicality and economics of the proposed procedure are recommended prior to implementation.

Application of the proposed bio-assay procedure to any given spacecraft material yields an upper bound estimate, $\bar{C}$, of an unknown mean concentration of buried organisms within the material. The usefulness of this estimate depends both upon its accuracy and the amount of information it contains. Acceptable quantitative measures of the effects of the previously indicated error sources on the accuracy of $\bar{C}$ must be determined. Further, the procedure must be shown to yield information which is needed and presently unavailable (e.g., estimates which are consistently greater than already known upper bounds are of little or no use). Attainment of these objectives requires the accomplishment of appropriate tests and analyses. For example, experimental test applications should be conducted on various classes of materials wherein the buried loadings are controlled (known). In addition, further analytical studies should be pursued which relate to the effects of (1) errors in the measurement of A_e and λ, (2) the assumption of uniformly distributed organisms and (3) the errors introduced by virtue of the fact that estimates relate to the mean concentration rather than the particular concentration going aloft in a spacecraft.

A research area requiring investigation is the engineering practicality of applying the proposed procedure. It must be determined, for example, whether appropriate control and measurement of the area exposed, A_e, is feasible. (The requirements on this accuracy should evolve from the efforts discussed in the preceding paragraph.) The practicality of appropriately culturing the exposed surface areas also requires additional study.

Finally, consideration must be given to the economics of the proposed procedure. For the most part, this should be based upon the cost of securing and processing sufficient numbers and varieties of sample spacecraft materials.

APPENDIX A

Computation of Confidence Intervals for Binomial "Success" Probability

APPENDIX A - Computation of Confidence Intervals for a Binomial "Success" Probability

Confidence intervals can be established for the "success" parameter of a Binomial distribution by using an approximating normal distribution. These intervals can then be transformed into upper and lower bounds upon the bio-load.

As indicated in the main body of this report, the confidence limits established for the mean bio-load are directly related to the confidence intervals for a Binomially distributed variable $\hat{p}$, which represents the observed proportions of times contamination was detected in the load estimation procedure. Expression (A1) on the facing page expresses the definition of a confidence interval. The interpretation of this expression is, for a confidence of $1-\alpha$, the probability that the parameter p is contained within the interval (p_L, p_u) is greater than or equal to $1-\alpha$. For the particular use of a Binomial distribution, Expression (A1) is solved by first considering expressions (A2) and (A3). In these expressions, the parameters p_u and p_L take on the largest possible value such that the given inequalities are satisfied. This would then give the $(1-\alpha)$ confidence interval of (p_L, p_u) on the parameter p. However, from a computational point of view, Expression (A2) and (A3) are subject to round-off errors and machine overflow. By using a well-known normal approximation to the Binomial distribution, we can replace Expressions (A2) and (A3) with the corresponding expressions resulting from this approximation. The approximating normal distribution will have mean p and standard deviation

$$\sqrt{\frac{p\ (1-p)}{s}}$$

Thus p and $\hat{p}$ are related as in Expression (A4) on the facing page. This equation must be solved for p in order to obtain p_L and p_u. It can be shown that Expression (A5) is the result of solving (A4) to obtain p in terms of $\hat{p}$. Expression (A4) with the plus sign corresponds to the upper half of an ellipse which gives the upper confidence limit on p. Likewise, using this expression with the negative sign gives the lower half of this ellipse corresponding to the lower confidence limit on p. A family of these ellipses is shown in Figures (I-B) thru (IV-B) of Appendix B. Since we are interested in upper bounds on the bio-load, Expression (A6) gives the upper bound on the bio-load in terms of p_u.

In the load estimation procedure a value for $\hat{p}$ would be obtained for the particular sample size used. For this value of $\hat{p}$ and for a desired confidence level, p_u would be obtained from Expression (A5).

$$P_r \left\{ p_L \leq p \leq p_u \right\} \geq 1 - \alpha \tag{A1}$$

$$P_r \left\{ \hat{p} > p_u \right\} = \sum_{x=sp_u}^{x=s} \binom{s}{x} p^x (1-p)^{s-x} \leq \frac{\alpha}{2} \tag{A2}$$

$$P_r \left\{ \hat{p} < p_u \right\} = \sum_{x=0}^{x=sp_L} \binom{s}{x} p^x (1-p)^{s-x} \leq \frac{\alpha}{2} \tag{A3}$$

$$\hat{p} = p \pm \delta \sqrt{\frac{p(1-p)}{s}} \tag{A4}$$

$$P_{L,u} = \frac{s}{s+\delta^2} \left\{ \hat{p} + \frac{\delta^2}{2s} \pm \delta \left[\frac{\hat{p}(1-\hat{p})}{s} + \left(\frac{\delta}{2s} \right)^2 \right]^{\frac{1}{2}} \right\} \tag{A5}$$

s = sample size

δ = standardized normal deviate

APPENDIX B

Graphical Displays of Confidence Limits

Figure I-B - 50% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)

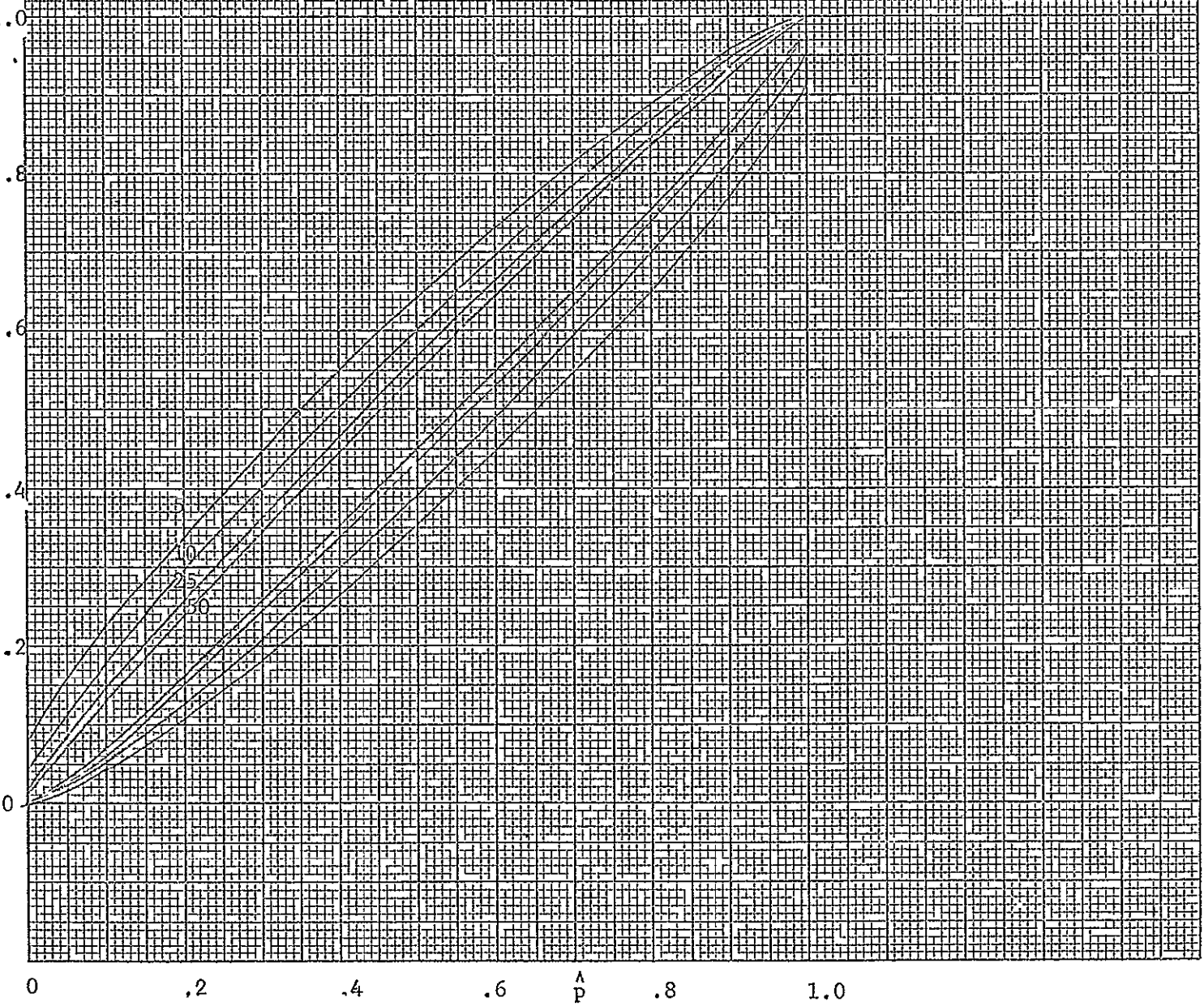


Figure II-B - 80% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)

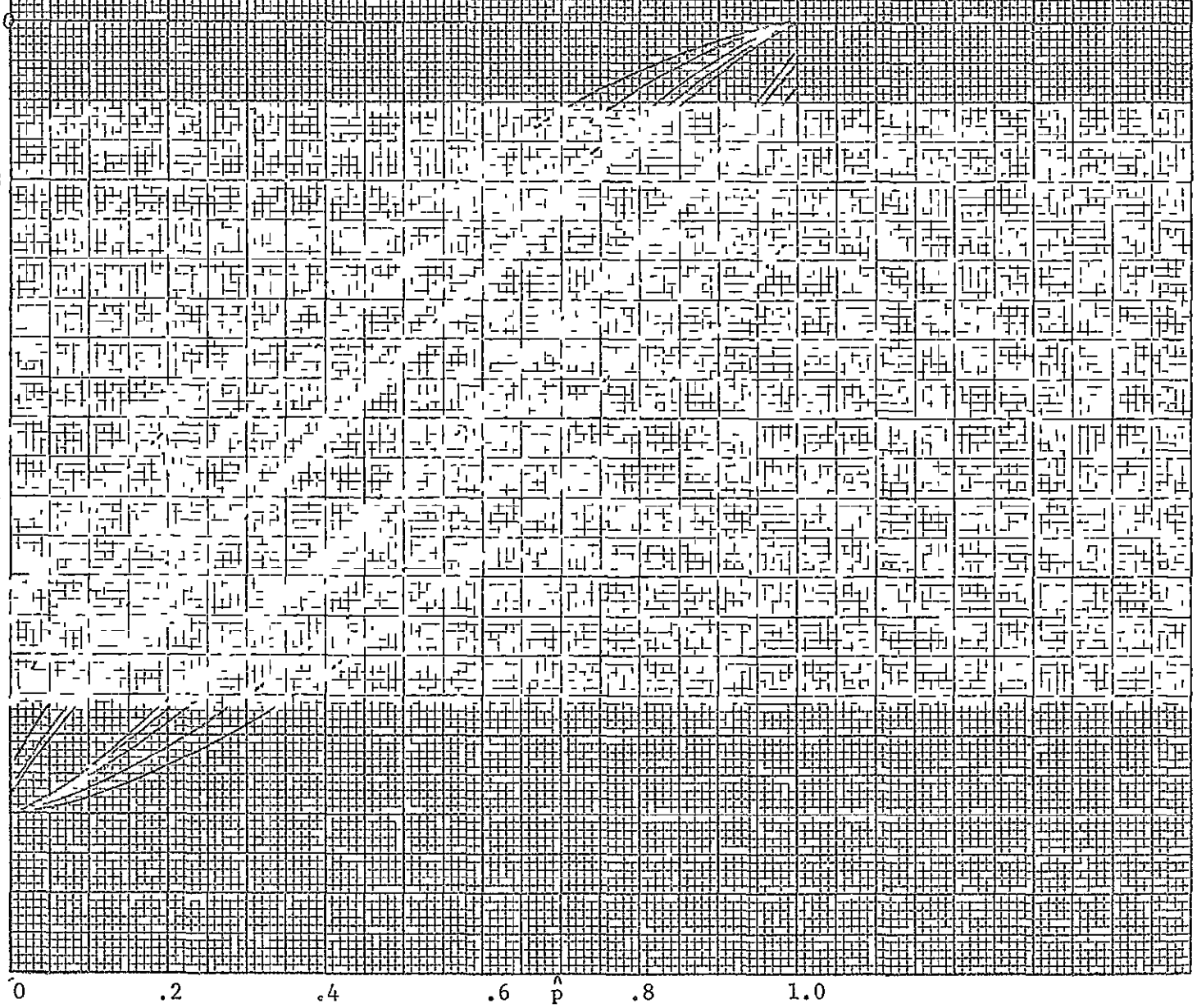


Figure III-B - 90% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)

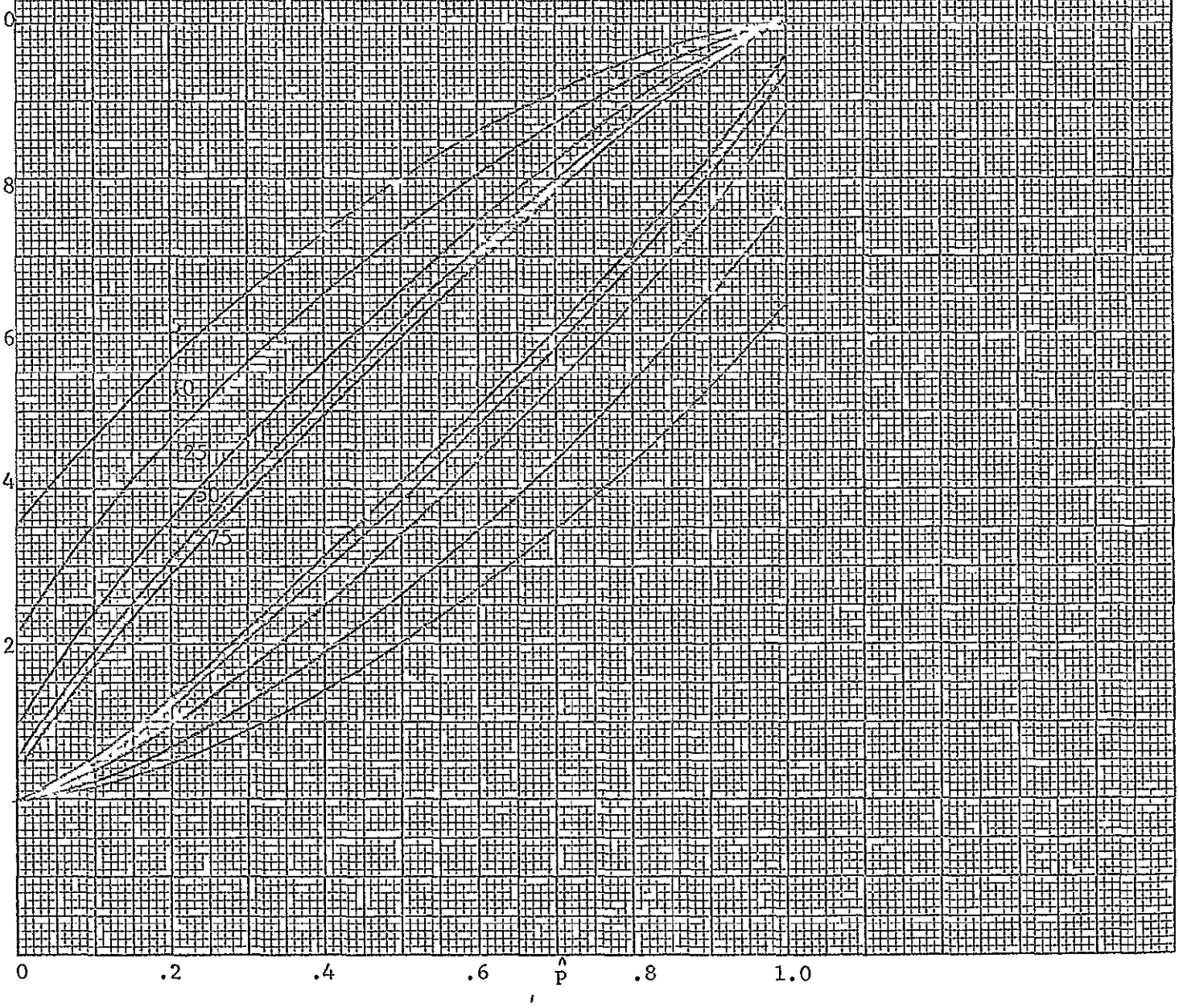
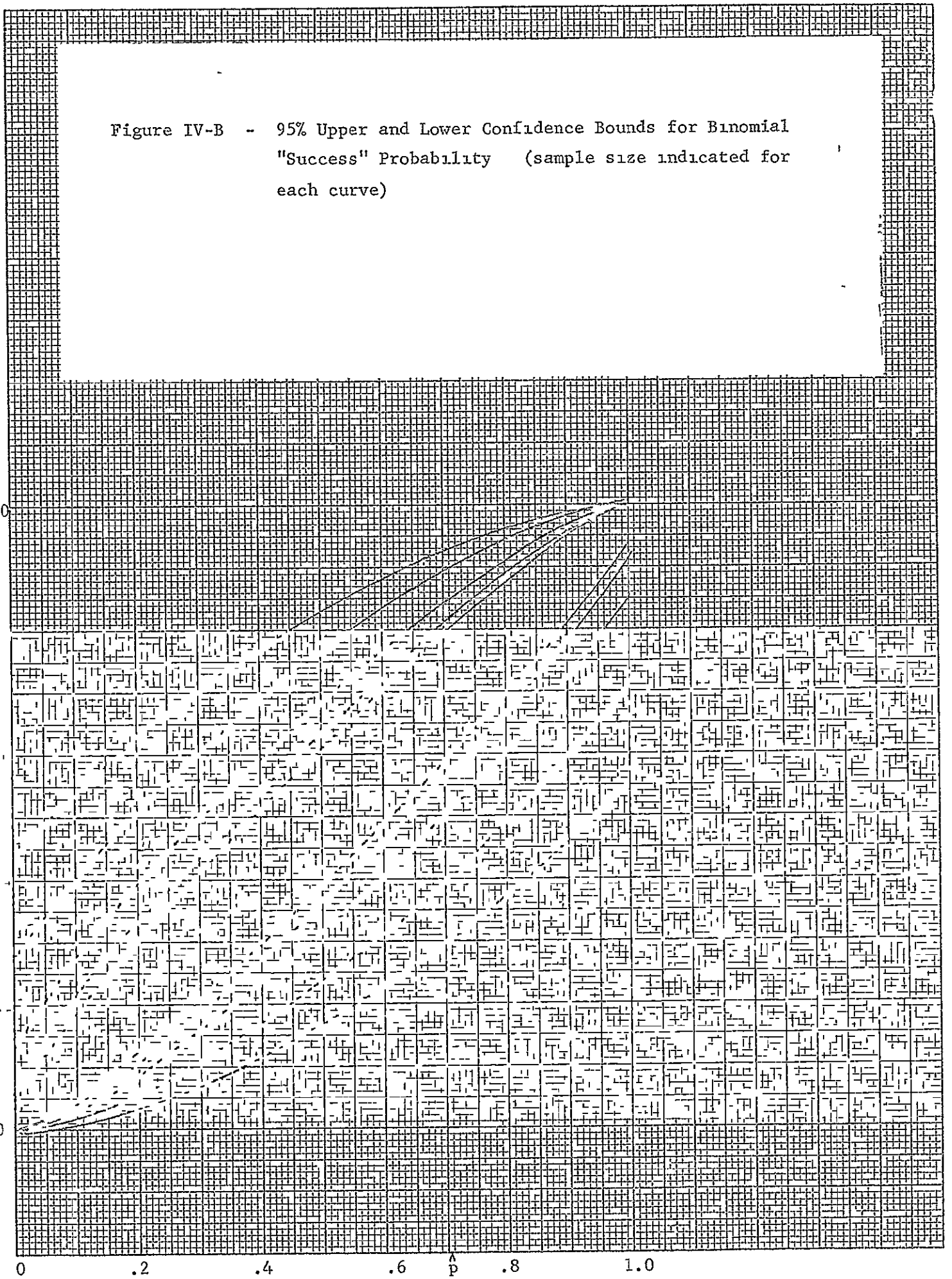


Figure IV-B - 95% Upper and Lower Confidence Bounds for Binomial "Success" Probability (sample size indicated for each curve)



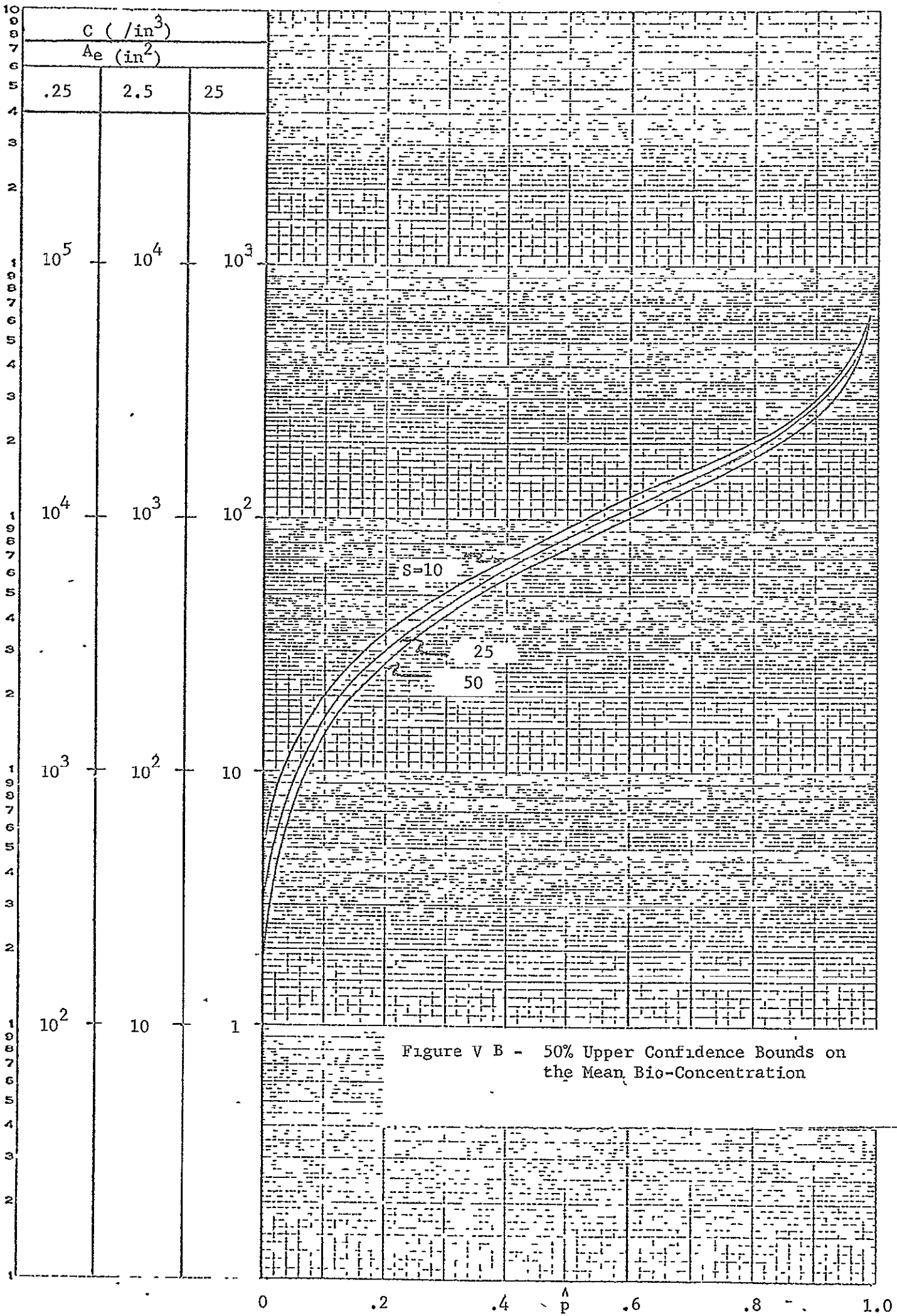


Figure V B - 50% Upper Confidence Bounds on the Mean Bio-Concentration

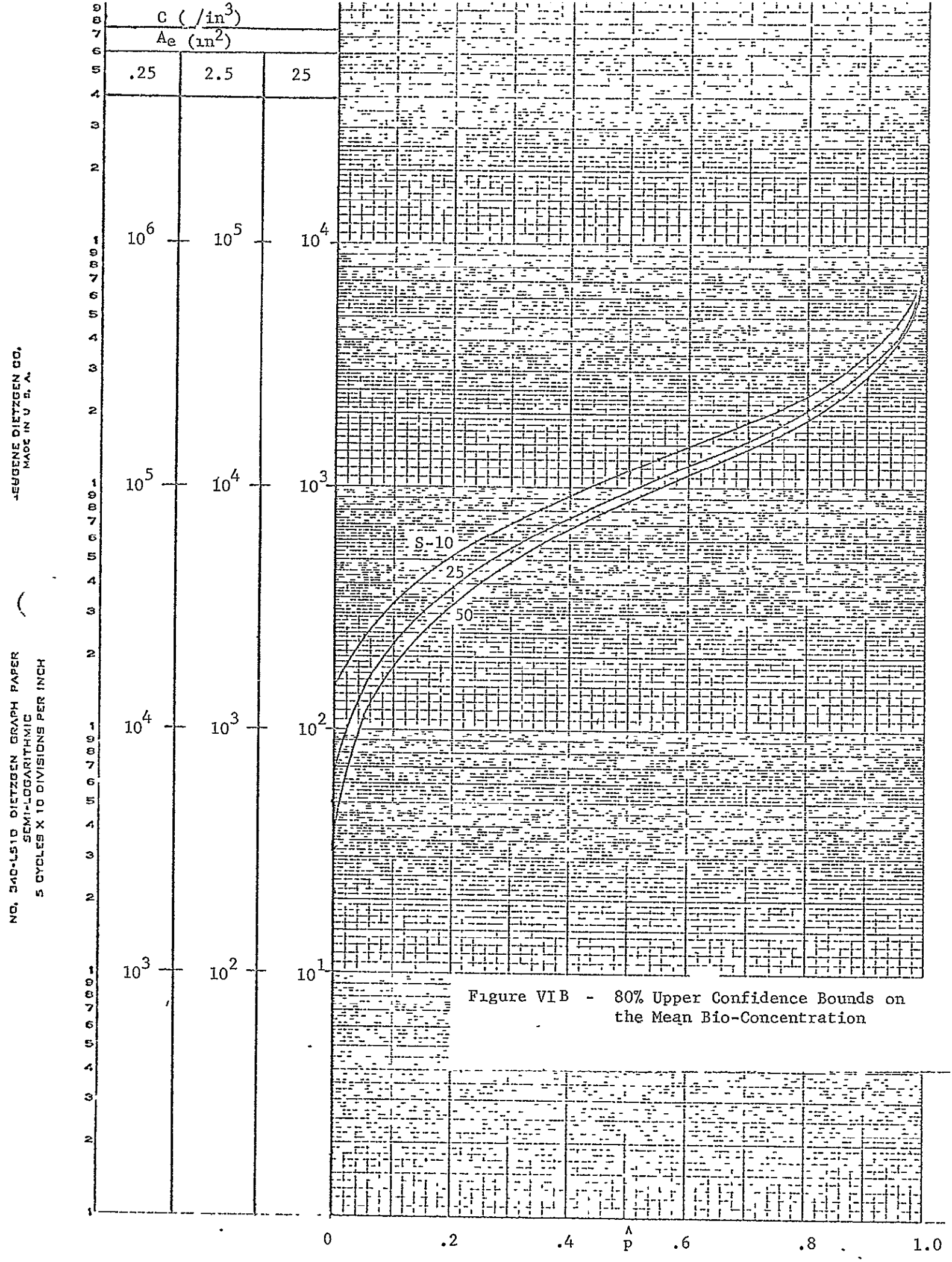


Figure VIB - 80% Upper Confidence Bounds on the Mean Bio-Concentration

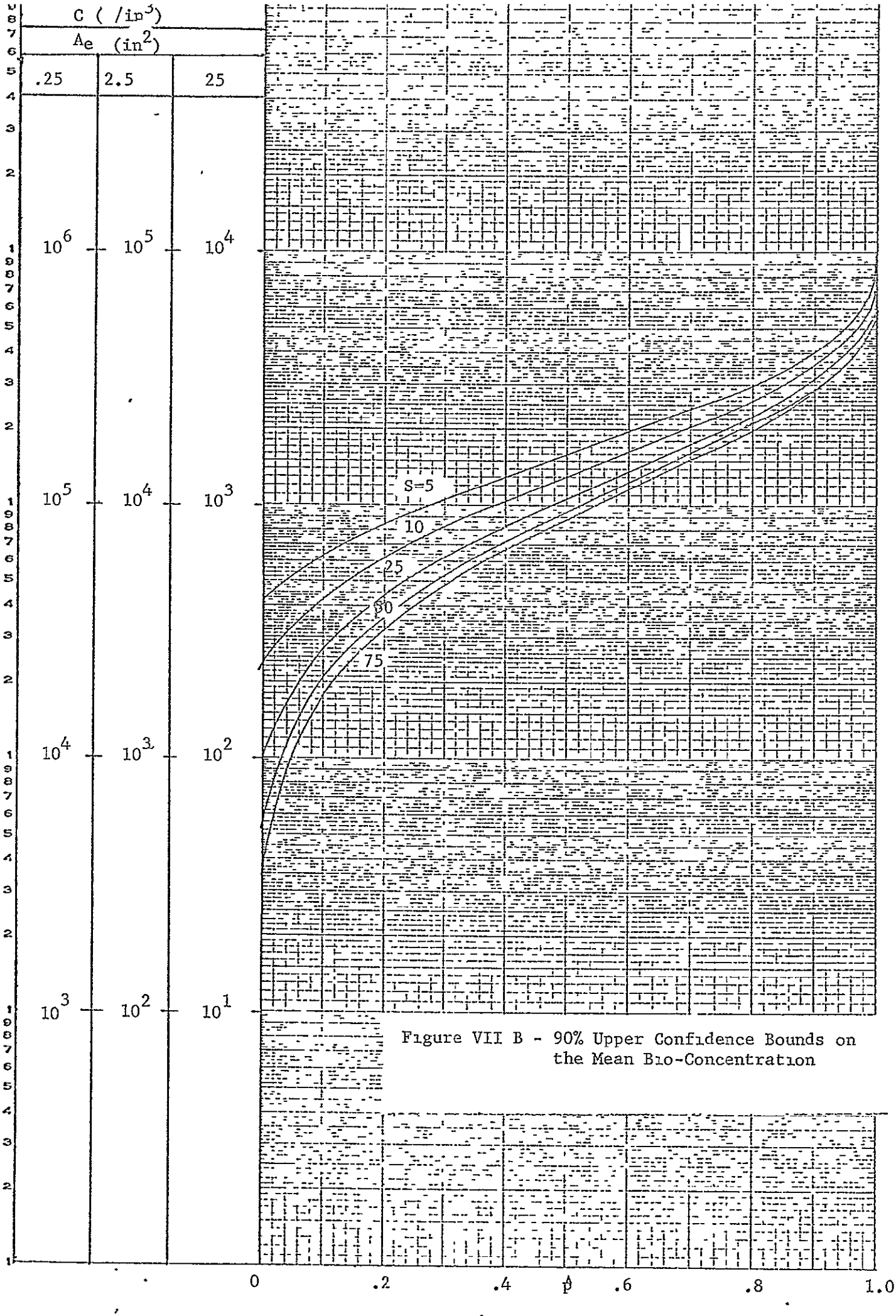


Figure VII B - 90% Upper Confidence Bounds on the Mean Bio-Concentration

REFERENCES

1 Schalkowsky, Samuel, Kline, Robert C., and DeGraff, Elliott Effect of Microbial Release Probabilities on Spacecraft Sterilization Requirements. Tech. Rept. SR-034, Systems Res. Div , Exotech Inc , Aug 1968.

2. Fraser, S J The Survival and Release of Viable Microorganisms from a Solid Material After a Hard Impact. Rev Ltr. D2-114143-1, The Boeing Co., n d

APPENDIX D

The Release of Buried Microbial Contamination by Aeolian Erosion

THE RELEASE OF BURIED MICROBIAL CONTAMINATION BY AEOLIAN EROSION

Prepared by

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J Lyndon Woodall

under

Contract NASw-1734
National Aeronautics and Space Administration

August 1969

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The Release of Buried Microbial Contamination by Aeolian Erosion

A. INTRODUCTION
   1. Brief statement of planetary quarantine objective
   2. Description of the physical process treated

B. THE EROSION PROCESS
   1. Derivation of $\Delta V/V$ for a sphere
   2. Numerical data for erosion rates of lucite, aluminum
   3. Numerical data for Martian wind velocities

C. PROBABILITY OF RELEASE
   1. Derivation of $P(\text{release} \mid \Delta V \text{ erodes in } t_q)$
   2. Sensitivities of $P(\text{release} \mid \Delta V \text{ erodes in } t_q)$

D. CONCLUSIONS AND RECOMMENDATIONS

DESCRIPTION OF THE PROCESS BEING TREATED

The impact of a lander on a planet could have serious consequences if one desires not to contaminate the planet. In the light of this one should examine the implications of fracturing and exposing of surfaces which might "instantaneously" or subsequently release viable spores. Here we will consider the relatively slow process of erosion although the fracture and the erosion phases are not necessarily independent. For our purposes we will take the fracture as having occurred and characterized by a fracture-ratio. (ref. #2) The fracture-ratio is defined as the area exposed through fracture divided by the volume of the sample. An expression for the erosion of spherical shaped particles and an expression for the probability of release given that a quantity of the sample erodes in the quarantine period have been derived. Calculations based on experimental data have been made for the erosion rate.

THE EROSION PROCESS

Assume no preferred wind direction and assume the fragments roll about freely. Then erosion of a fragment by wind-borne agents is often radially symmetric. This suggests that we can study the erosion of a sphere as typical of the erosion process.

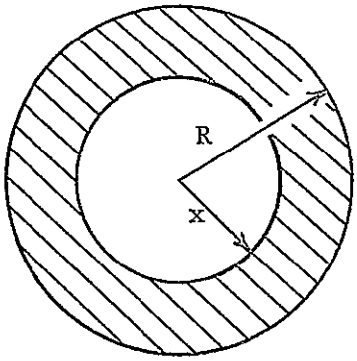


Figure 1. The geometry which will be considered here.

The ratio of volume removed to original volume of the fractured piece is

$$\frac{\Delta V}{V} = 1 - \left(\frac{x}{R} \right)^3 \qquad (1)$$

(case 1) $x = R - \epsilon tq$ if $R \geq \epsilon tq$

or (case 2) $x = 0$ if $R < \epsilon tq$

where ϵ = erosion rate (rate of eroding surface) (ref. #2)

tq = time of quarantine.

when $R \geq \epsilon tq$, Equation 1 becomes $\frac{\Delta V}{V} = 1 - \left(1 - \frac{\epsilon tq}{R} \right)^3$ (2)

The equation in this form is unmanagable due to the fact that the R parameter is still present. We would like to find R as a function of a macro-parameter, say the fracture-ratio f as previously defined (ref. #2). To do this it is necessary to assume some break-up model (i.e the way R is related to the dimensions of the original sample of material)

Suppose the original sample were a cube of edge length L, and fractures completely into equal sized particles of which a sphere of radius R is typical. The number of particles is approximately

$$N = \frac{3L^3}{4\pi R^3} \tag{3}$$

and the fracture ratio is $f = \frac{S_f - S_1}{V} = \frac{N4\pi R^2 - 6L^2}{L^3}$

where S_f is the final surface exposed and S_1 is the initial surface. Equation (3) simplified gives,

$$\frac{1}{R} = \frac{f}{3} + \frac{2}{L} \tag{4}$$

Equation (2) then becomes $\frac{\Delta V}{V} = 1 - \left[1 - \epsilon t q \left(\frac{f}{3} + \frac{2}{L} \right)\right]^3$ (5)

The erosion rate (ϵ) can be determined from existing data. In experiments by Neilson and Gilchrist (ref. #3), lucite and aluminum were eroded by aluminum oxide particles of 210 micron diameter. The results show that

$$\frac{dm}{dt} = E(\theta) \cdot \phi \cdot A \tag{6}$$

where $\frac{dm}{dt}$ = the rate of loss of mass

$E(\theta)$ = a proportionality factor depending upon the angle of attack and the material.

ϕ = the mass-flux of particles per unit area striking the surface.

A = the cross-sectional area

The mass eroded from the sphere in Figure 1. is

$$\Delta m = d\Delta V = d\frac{4\pi}{3}(R^3 - x^3) \tag{7}$$

where d = density of substance being eroded

Differentiating (7) with respect to time and combining with (6) gives,

$$e = \frac{dx}{dt} = \frac{E(\theta) \cdot \phi}{2d} \tag{8}$$

Substituting equation 8 into equation 5 gives,

$$\frac{\Delta V}{V} = 1 - \left[1 - \frac{E(\theta) \cdot \phi t q}{2d}\left(\frac{f}{3} + \frac{2}{L}\right)\right]^3 \tag{9}$$

ESTIMATION OF PARAMETERS

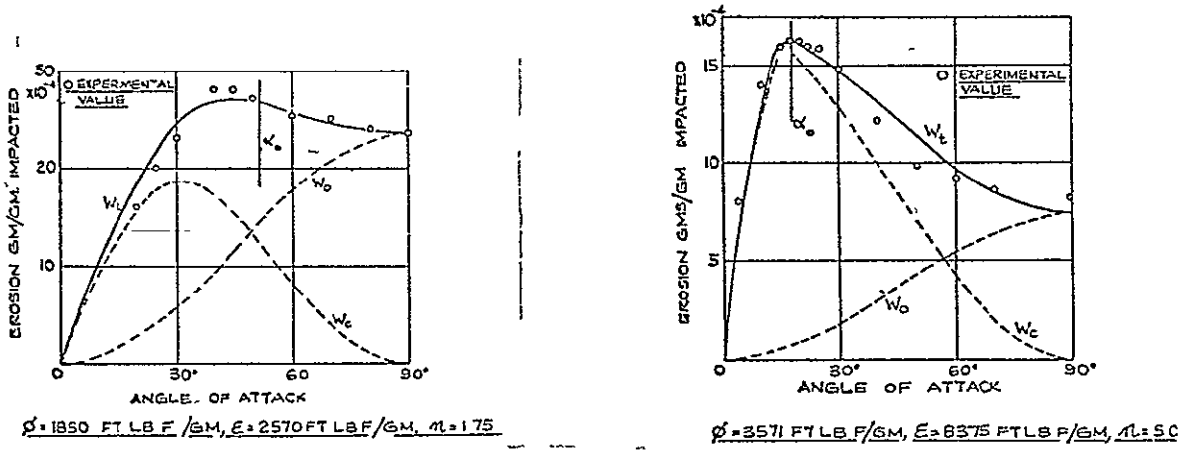
Hertzler at McDonnell Aircraft Corporation in simulation of the Martian atmosphere has arrived at estimates of velocities of winds on Mars and volume of abrasives carried by the wind (ref. 4)

Average wind velocity = 220 fps.

Particle concentration = 10^{-4} oz./ft^3

Then the mass-fluence per unit area is,

$$\phi = 4.68 \times 10^{5} \text{ lb/M}^2\text{/YR.}$$



Ø = 1850 FT LB F/GM, E = 2570 FT LB F/GM, m = 1.75

Ø = 3571 FT LB F/GM, E = 8375 FT LB F/GM, m = 5.0

Figure 2 (after Neilson and Gilchrist) ref. #3

(a) Erosion vs. angle of attack characteristic for lucite eroded by 210μ aluminum oxide particles at 420 fps.

(b) Erosion vs. angle of attach characteristic for aluminum eroded by 210μ aluminum particles at 220 fps.

From figure 2a one may estimate E (Ø). We will take the maximum of the curve for lucite for our calculation. (i.e. $E(\theta) = E = 2.5 \times 10^{-3}$)

Note, however, that the experiment for lucite was conducted for particles at 420 fps where as we are postulating wind and particle velocities averaging 220 fps. A corrected estimate of E is taken to be

$$E = 2.5 \times 10^{-3} \left(\frac{220}{420} \right)^2 = 6.9 \times 10^{-4}$$

For lucite $$d = \frac{1.3 \times 62.4}{(.305)^3} = 2.9 \times 10^3 \ \text{lb/M}^3$$

or the erosion rate for lucite is $$\epsilon = \frac{E \cdot \emptyset}{2d} = 5.6 \times 10^{-2} \ \text{M/YR.} \qquad (10)$$

A similar calculation for aluminum yields $$\epsilon = 1.9 \times 10^{-2} \ \text{M/YR.}$$

MODEL OF THE PROBABILITY OF RELEASE

Assume a cube of edge length L impacts and fractures with fracture-ratio f. Also, assume that ΔV of the original volume V is eroded away during the period of time tq. If M spores are distributed randomly in the volume V then

$$P(\text{release} \mid \Delta V \text{ erodes in tq}) = 1 - e^{-M\frac{\Delta V}{V}} \qquad (11)$$

Substituting equation 10 into equation 9 and combining with equation 11 gives an estimate of the probability of release

$$P(\text{release} \mid \Delta V \text{ erodes in tq}) = 1 - e^{-M\left\{1-\left[1-\frac{E\emptyset tq}{2d}\left(\frac{f}{3}+\frac{2}{L}\right)\right]^3\right\}} \qquad (12)$$

SENSITIVITY OF P (re/ΔV erodes in tq) TO ε, f, AND L

We calculated the following two cases.

PARAMETER	BEST	WORST
ε	10^{-6} m/year	10^{-2} m/year
f	10^{2} m^{-1}	10^{5} m^{-1}
L	1 m	10^{-2} m

Graphs Figure 3a and 3b are the results of the calculations. In both graphs the model indicates virtually no dependence on L, the size of the sample, and only when all parameters are "near" best case does dependence on ε, f become important. In other words, the amount of material eroded from the spacecraft debris, after a hard impact, is almost independent of its size but does depend on the fracture ratio f due to impact, and the erosion rate ε of the materials present. A further observation can be made if the quantity

$$\varepsilon t q \left(\frac{f}{3} + \frac{2}{L} \right) << 1$$

then Eq. 12 becomes $P (re/\Delta V) = 1 - e^{- M \varepsilon t q (f + \frac{6}{L})}$ (13)

furthermore if $M \varepsilon t q (f + \frac{6}{L}) << 1$

then expansion of the exponential gives the approximation $P (re/\Delta V) = M \varepsilon t q (f + \frac{6}{L})$ (14)

Note the similarity between Eq. 14 and the model of $P(re/V_I)$ in reference No. 1 if one makes the following transformations

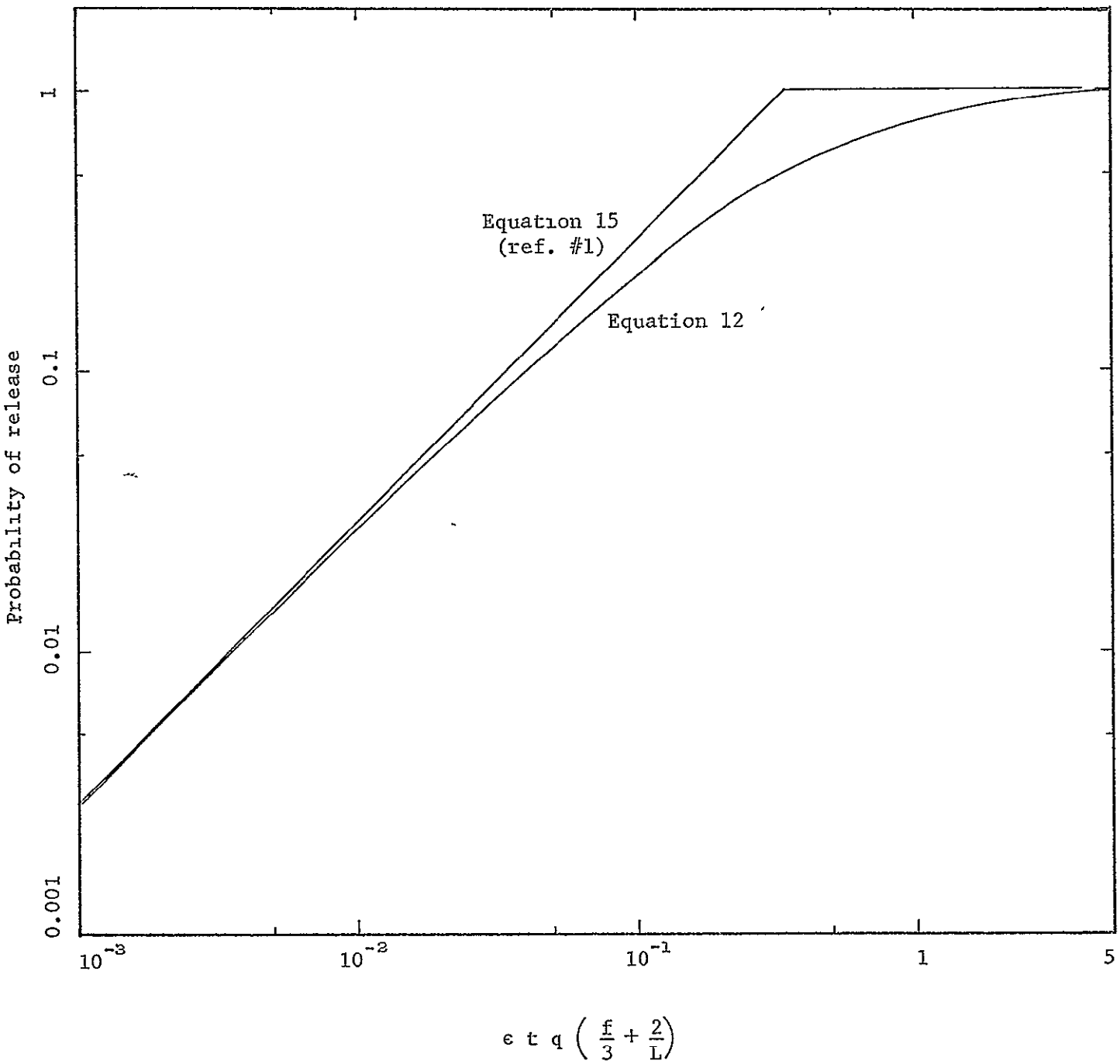
$$f^{(o)} \longrightarrow \frac{6}{L} = \frac{6L^2}{L^3}$$

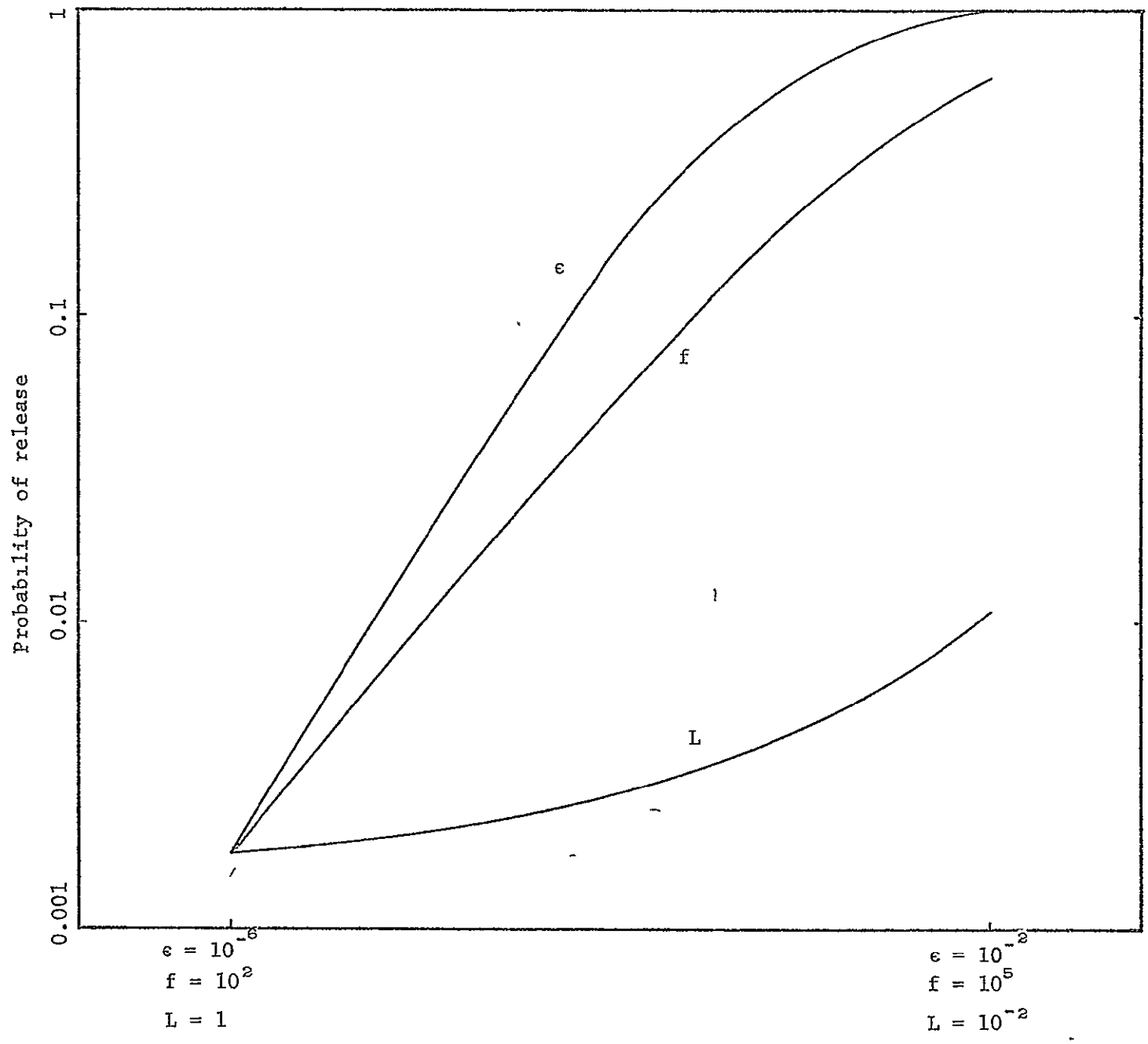
$$f \longrightarrow f$$

$$M(o) \longrightarrow M$$

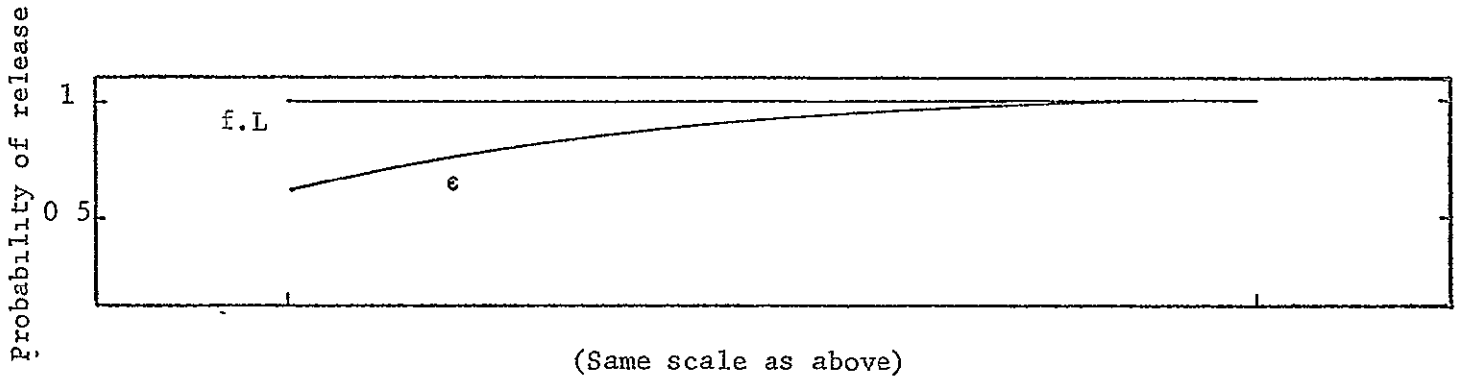
i.e. $$P(re|\Delta V) = M(o) \ etq \ (f^{(o)} + f) \qquad (15)$$

Figure 4 shows the error involved in using equation 15 instead of equation 12 as a function $etq \ (\frac{f}{3} + \frac{2}{L})$ if M is on the order of 1.


Probability of release
1
0.1
0.01
0.001
Equation 15
(ref. #1)
Equation 12
10^{-3}
10^{-2}
10^{-1}
1
5
$\epsilon\ t\ q\left(\frac{f}{3}+\frac{2}{L}\right)$



3a. All "best" case except for variation of one parameter



(Same scale as above)

3b. All "worst" case values except for variation of one parameter.

CONCLUSION AND RECOMMENDATIONS

Curves 3a and 3b indicates that the dimensions of the original sample are not of primary importance in estimating the P (release | ΔV erodes in tq) .

On the basis of this model the erosion rate ϵ and the fracture ratio (f) show no clear domination one over the other for the range of parameters considered. There is difficulty in estimating both but there is more uncertainty about ϵ. The range chosen for ϵ is 10^{-2} M/yr. to 10^{-6} M/yr. This involves 4 decades and includes the totally "worst case" situation in which the eroding agent is assumed to be uninterpreted at a rate of 220 feet per second for 17 years! The other extreme is the order of magnitude of terrestrially observed erosion rates. This wide range of uncertainty arises from our uncertainty about the flux of the eroding agent, $\emptyset$. It is recommended that a closer examination be taken of $\emptyset$ with a view to arriving at an estimate of the expected abrasive-flux. This might possibly be done by examining the distribution in crater populations of the moon and Mars as photographed by Mariner's V, and VI to arrive at a better estimate of $\emptyset$ for Mars. Because of the scarcity of small craters on Mars (Ref. #5) estimates of the extent of the transport-erosion process can be made.

REFERENCES

1. Exotech, Inc., Systems Research Division, "Development and Application of a System Model for Spacecraft Sterilization", Final Report Contract NASw-1558 for NASA, August 1969.

2. S. Schalkowsky, R. Kline, E. DeGraff, "Effect of Microbal Release Probabilities on Spacecraft Sterilization Requirments", Interim Report Contract NASw-1558 for NASSA, Office of Planetary Quarantine, August 1968, EXOTECH, INC.

3. Neilson and Gilchrist, "Erosion by a Stream of Solid Particles" WEAR Vol II 111-122 Feb. 1968.

4. G. Dyhouse, "Simulated Martian Sand and Dust Storms and Effects on Spacecraft Coatings", ASTM/IES/AIAA Second Space Simulation Conference (Sept. 1967). Am. Soc. Testing Materials, 1967 Philadelphia, Pennsylvania.

5. Leighton, et al. Mariner 6 and 7 Television Pictures Preliminary Analysis, Science Vol 166 No. 3901 Oct. 3, 1969.

APPENDIX E

Implementation of a Chemical Contaminant Inventory for Lunar Missions

IMPLEMENTATION OF A CHEMICAL CONTAMINANT INVENTORY FOR LUNAR MISSIONS

Prepared for

National Aeronautics and Space Administration
Office of Bioscience Programs

Contract NASw-1734

December 1969

by

EXOTECH INCORPORATED
Systems Research Division
525 School Street, S.W.
Washington,D.C. 20024

TRSR 70-07

I. INTRODUCTION AND SUMMARY

This report summarizes the study conducted by Exotech Incorporated under Task D of the subject contract. This task was initiated under modification No. 2 to the contract dated August 15, 1969.

The study described herein represents a follow-on effort to the planning study conducted by Exotech Incorporated under an earlier contract aimed at selecting an approach to establishing an organic constituent inventory for the Moon[1]. Based on the results of this planning study NASA selected an approach containing the following two essential guidelines:

(a) Documentation of lunar mission spacecraft should be preserved for possible future examination to identify types and quantities of organic materials deposited on the Moon. A detailed analysis of this documentation should not be undertaken until justified by requirements from investigation of lunar sample materials.

(b) An information system should be established which identifies regions of the Moon where the risk of contamination in surface samples is significant, including identification of the degree of risk and the spacecraft which contribute to the location - dependent material contamination.

The present study represents the initial step in the implementation of the above approach in that it considers the detailed procedures and tasks to be undertaken to collect, evaluate, store and disseminate data which will serve anticipated needs of lunar sample investigators, consistent with the requirement that costs associated with implementation and operation of the inventory be consistent with known needs for this information. The primary tasks pertinent to this effort involved (1) determining the availability of lunar mission vehicle documentation and the means for collecting it in a form suitable for future evaluation, (2) the collection and

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[1] Planning Study for an Organic Constituents Inventory Program, R.G. Lyle, Exotech Incorporated, Report No. TRSR-68-029 under Contract No. NASw-1666, May 1968

utilization of spacecraft trajectory parameters, landing sites and dispersion patterns for crash and soft landings, and (3) evaluating the compatibility of required date inputs with the existing Planetary Quarantine information system.

Sections II and III of this report summarize, respectively, the approach taken in this study and the detailed analysis of the questions considered. This is followed in Section IV by a set of recommendations for implementing the chemical materials inventory in accordance with the guidelines set forth above. A summary of these recommendations is also provided below.

.The minimum requirements for the preservation of pertinent materials information are retention of documentation by lunar mission spacecraft contractors and notification of the Planetary Quarantine Office prior to disposal. This would apply to NASA contractors for the Ranger, Lunar Orbiter, AIMP, Surveyor and Apollo programs. In addition to this, however, the following procedures are recommended for consideration by NASA.

1. Designate a Federal Records Center to receive all documents and organize a filing system by mission designations.

2. Order all documentation sent by U.S. Mail to the designated Federal Records Center at end of the existing contractual requirements for retention, including those documents already in other records centers.

3. When contractors and agencies desire to retain copies of the information, require film copies suitable for aperture card insertion to be forwarded to the designated center.

4. Expand COMAT/TRIS System of Apollo program to include materials information on module sections not now covered. Information is not to include flammability or other data as in current system, but should include specification and property information which would be of use in identifying portions of the material at a later date.

5 Require copies of documentation on future flights to be supplied within thirty days after launch.

Concerning the preparation of an information system, a two step procedure is recommended as follows.

1. Prepare a contamination risk model of the lunar surface either in tabular form or preferably as a "risk-contour" map, based on the best available estimates of spacecraft landing sites and the particle dispersion associated with the mass and velocity characteristics of hard and soft landers. Dispersion models suitable for this purpose are the Sandia and Grumman models referred to in the text.

2. Disseminate among lunar sample investigators, and to others associated with the planning of lunar scientific exploration information concerning the documentation to be kept in storage and the available information. Include a questionnaire to permit the estimation of expected usage of information systems data of various levels of detail.

The above recommendations are expected to result in an optimum procedure for the implementation of a lunar chemical contaminant inventory, consistent with known cost and technical constraints. These recommendations and the source data upon which they are based, are elaborated upon in the body of this report.

The study described herein has been conducted by Robert G. Lyle and Lester D. Shubin of Exotech's Systems Research Division, under the overall direction of Samuel Schalkowsky. The contributions of highly pertinent information by the various personnel cited in this report from NASA, NASA contractors and from the scientific community, are gratefully acknowledged.

II. APPROACH TO THE STUDY

1. Definition of the Problem

The proper application of the results of this study should make possible the preservation of space vehicle documentation from which contaminant identification and quantization may be developed if required, and should enable the delineation of contamination risk zones on the lunar surface.

In order to gain an in-depth understanding of the entire problem, it was subdivided into separate tasks. These tasks define the inputs required to accomplish the goals set:

a. Determine the location and form of the documentation required to identify spacecraft materials.

b. Examine the available dispersion models developed for high speed impact and soft landing vehicles and determine their applicability.

c. Determine the most productive course to follow in the collection and storage of pertinent documentation.

2. Procedures Followed in the Investigation

Personal communication was established with the cognizant personnel (see Table 1) at the agencies and companies holding the documentation. Discussions covered the availability of the documentation, the amount, the current form, its storage location, and the length of time that it would be retained. These conversations were followed by written correspondence which formalized estimates by these companies of the costs of copying, transferring and sorting the documentation in several ways. These alternatives are presented within this report.

Documents were acquired from the Grumman Aerospace Corp., detailing the dispersion model for contaminants emanating from soft landers and from the Sandia Corp., relating to both the dispersion of fragments from hard landers and the Planetary Quarantine Lunar Programs Information System. These documents were examined to ascertain the applicability of the dispersion

TABLE 1

PROJECT	NAME OF CONTACT	ORGANIZATION
Ranger	Mr. Irl Newland,* Librarian	JPL
Surveyor	C. W. Lefever, Mgr ** Contracts, SSD	Hughes Aircraft
Orbiter	H. M. Miller, Mgr *** Contracts Space	Boeing
Apollo	Arlie Carter J. Steinthal	GE/Houston NASA/MSC
AIMP	F. Ledoux	GSFC

*For Technical Information

* Mrs. V. Pritchard (213-354-4321)

** Perry Ackerman (213-648-4134)

*** W C. Galloway (206-656-2121)

models to the inventory problem and to define the interface between the existing information system and the inventory. In addition to this literature study, personal communication was established with a number of personnel concerned with the content of these documents, and with related problems of lunar surface contamination.

In addition to the personnel listed in Table 1, conversations were held with the following persons:

1. Mr. A.L. Roark of Sandia Laboratories on the Lunar Information System computer program;
2. Dr. M. Aronowitz of Grumman Aerospace Corp., on the dispersion model of the LM exhaust products;
3. Dr. A.L. Burlingame, Space Sciences Laboratory, Univ. of Calif., on the sensitivity of mass spectrometric measurements of lunar materials;
4. Dr. Elbert King, Curator of the Lunar Receiving Laboratory, concerning current practices of sample preservation,
5. Dr. P.R. Bell, Manager of the Lunar Receiving Laboratory, concerning possible need for the inventory;
6. Dr. Donald Flory, Gas Analysis Laboratory, LRL, to discuss LM exhaust products, and possible contamination in the Surveyor 3 crater,
7. Dr. I. Adler of the Theoretical Branch at GSFC, to explore possible needs of the inorganic chemistry investigators.

Communication was also established with the Data and Tracking Group at JPL to obtain the impact location of Orbiter IV, and with the National Geographic Society in order to determine the accuracy and sources of information used for placement of the impact sites of the USSR vehicles on their Moon chart.

The Micromation Co., of Washington, D.C. supplied estimates of the cost of various methods of copying documents.

When all of the information was at hand, the alternatives were studied and conclusions were reached concerning the procedures which would accomplish the intended goals in the most effective manner. Recommendations have been developed on the basis of these conclusions which will enable the cost effective implementation of the previously selected inventory approach, mentioned in the Introduction and described in detail in Exotech's previous report[2].

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[2] Ibid.

III. ANALYSIS

A. Current Status of Documentation

Ranger: The current status of the Ranger Project documentation, the earliest of the programs under study for this report, has been reasonably well defined. All documentation currently existent is stored under the cognizance of Mr. Irl Newland, Chief Librarian, and his assistant, Mrs Vivian Pritchard, of the Jet Propulsion Laboratory. The bulk of the material is stored at the Federal Records Center, at Shelly Air Force Base. The documentation is supposed to be reviewed every three years for possible disposal, but to date this has not been done, and the Exotech investigator was told that it will be stored indefinitely. Some documentation continues to turn up in various files in JPL offices and is sent to the library when it is found.

Most of the Ranger documentation is on 16 and 35mm film and much of it, especially the drawings, is stored on aperture cards for easy retrieval. Good estimates of the amount of documentation involved in the Ranger project have been difficult to obtain, but the best available indications are that approximately 10,000 documents and drawings are contained within the files.

Lunar Orbiter: Boeing Aircraft Co., Seattle, Washington is contractually obligated to retain Lunar Orbiter spacecraft documentation for the following time limits: technical data-3 years, financial data-10 years. In each case the period begins from the time of the official termination of the contract. It should be noted that parts lists for the Orbiter spacecraft are contained within 13,000 drawings and identify the applicable specifications which are on file at the Department of Defense Information Center, where they are available to NASA.

Surveyor: The Surveyor spacecraft was fabricated in two configurations. Surveyors I through IV are designated Group A; Surveyors V through VII are Group B. There are sufficient differences between the two configurations to warrant this division, which results in an increase in the amount of documentation required to completely describe the spacecraft. The documentation system used by the contractor, Hughes Aircraft Company, permits reproduction of both sets of data.

The documents pertaining to the Surveyor vehicles are currently held by the Hughes Aircraft Co., El Segundo, California in the form of films, aperture cards, and paper copies. Most of the technical data is recorded and stored on aperture cards which are of the punched card type containing a microfilm insert, coded for retrieval with the document identification such as the drawing number. The coding can be extended at any time to include such other identification as is needed.

The Hughes Aircraft Co. estimates that it has approximately 3500 aperture cards in its possession relating to the Surveyor project. It is likely that some additional documentation exists that Hughes has not included, since this is a surprisingly low quantity in comparison with the Boeing Company estimate for the Lunar Orbiters. However, the additional number of documents is not expected to be large and the estimate by Hughes Company is considered acceptable for the purposes of this study.

Apollo There are some 600 major contractors and numerous sub-contractors and suppliers for the Apollo program. They have produced thousands of drawings and documents, many of which are pertinent to the identification of chemical contaminants placed on the Moon.

Discussions with Mr. Gerald White at the Grumman Aerospace Corp., Bethpage, N.Y., revealed that the General Electric Corp. Houston office is the operating contractor of the COMAT System for NASA on the Apollo project. COMAT is an acronym for "Characteristics of Materials". The COMAT system is a computerized central data bank used in the recording and control of the use of non-metallic materials in the crew bays of the Apollo spacecraft. It is designed for the storage and retrieval of data on the use, status and characteristics of non-metallic materials considered for application in the manned spacecraft. The usage data consist of an accounting of materials in terms of their locations in the spacecraft and quantities and the functional requirements of their application. The status data consists of evaluation of the material safety and habitability in its application in terms of such parameters as combustion rate, fire point, odor, and carbon monoxide emission. The characteristics data include selected elements of flammability and outgassing test data.

The documentation used to supplement the COMAT data system is handled by the TRIS system (Test and Reliability Information System)[3]. This is a specialized document acquisition, storage and retrieval system, using automatic data processing to provide multiple listings and cost-indexing. TRIS acquires, microfilms, stores, retrieves and distributes documentation required to assist the reliability and quality control groups in their evaluation of certain parts, materials, and other hardware considered for use in the Apollo Program.

AIMP. An examination was made of the status of the documentation of AIMP D and E at the Goddard Space Flight Center. Mr. Frank LeDoux, of the Project Office, estimates that approximately two cubic feet of paper documentation including photographs exists. The documentation contains lists of materials, test results, and manufacturers specifications on much of the spacecraft materials. It is currently maintained in loose leaf folders and stored in a filing cabinet by Mr. LeDoux at GSFC.

The photographs are a necessary input to the inventory since they are keyed to the test programs and the identification of spacecraft parts would be difficult without them.

According to Mr. LeDoux, identification of materials used in experiment packages would not present a difficult problem. This is due to the fact that his records are virtually complete, and he knows the location of the additional data required.

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[3] General Electric, TRIS User's Manual, Houston, Texas

B. Alternative Methods for Preserving the Documentation

At this time, it is possible to retrieve the bulk of the documentation relating to the U.S. missions which have impacted or soft landed spacecraft on the Moon. However, it may be seen from Table 2 that the ends of the contractors' retention periods for the technical data are in the very near future. This imposes a constraint on the time available for implementation of the documentation collection required for the selected inventory model.

The alternatives available to the Planetary Quarantine Office under the specifications of alternative (d) of the previous Exotech report[4] requiring the preservation of documentation of all lunar contact missions are in increasing order of complexity:

1. Extend documentation retention agreements with the contractors pending a later decision

The simplest solution to the problem of preserving the documentation is to extend the retention agreements until at least 1975 and postpone a decision on the disposition of the documentation until then. This will gain time needed to determine the full extent of the requirements of the investigators.

The primary advantage of this alternative lies in its simplicity, and relatively low cost. The disadvantages are many, including the fact that the documentation is not readily available for examination by persons other than the contractors. In addition, it will be widely dispersed throughout the country, making it extremely difficult to gain any meaningful information from the documentation without considerable travel and time investment.

2. Direct contractors to transfer documentation to a designated Federal Records Center when the retention time expires

Under this alternative, the Government would require

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[4] Lyle, Loc. Cit.

TABLE 2

PROJECT	CUSTODIAN OF DOCUMENTATION	APPROXIMATE NUMBER OF DOCUMENTS	CONTRACTOR RETAINS DOCUMENTATION UNTIL
Ranger	JPL	10,000	1970 (?)
Surveyor	Hughes	3,500	1973**
Orbiter	Boeing	13,000	1977**
Apollo	GE/Houston	unknown	1975**
AIMP	GSFC	4,000	Indefinite Project continuing

* Analysis not needed-document call outs included

** Estimated close of contract

(?) Estimated

the contractors to transfer the documentation as it exists to a designated Federal Records Center at the termination of the retention period. The Planetary Quarantine Office would be notified of any impending changes in the location of the record material.

The advantage of this alternative is that it requires virtually no expenditure of funds. There are no storage or handling charges, and no transportation charge to NASA if the U.S. mails are used for shipping. In addition, the documentation eventually will all be located in a single storage center.

The main disadvantages are the time delay involved in transferring the documentation to the Government and in the variety of forms which must be handled.

3. Copy documentation in any form and send to designated Federal Records Center

Under this alternative, the contractors would submit duplicates of the documentation in whatever form is convenient to a selected Federal Records Center prior to a specified date.

The principal advantage gained is that the documentation will be transferred to a single Government operated facility at an early date, permitting greater accessibility than if kept only by the contractors.

The primary disadvantage of this alternative is that a variety of documentation forms must be dealt with. Retrieval of information from a collection of such diverse inputs would be difficult and costly.

4. Copy documentation into a suitable form for present and future use and store in a designated Federal Records Center

The significant gain in this alternative is the uniformity in the format of the stored documentation. The use of a

single type of copy will result in a time saving in handling the material during any subsequent search and retrieval over that in the other alternatives. The preferred format would be one which could be used in an aperture card at a later date if desired.

This procedure will cost more than the others initially, but may save money in the long run if information retrieval requests are expected. It is not necessary to process the data into a final form, only into a form which may later be processed into final retrieval form. This may be accomplished by converting the documentation into microfilm which could be entered into an aperture card system at a later date.

If the decision is made to store all lunar contact spacecraft documentation, a single Federal Records Center should be designated as the repository rather than utilizing the Federal Records Center nearest the present location of the documentation. If the U.S. Mail is used for shipment of the documents, the cost of transportation to NASA can be neglected. It would therefore be more beneficial to collect the documentation at a central point. From the point of view of convenience, this should be in or near the Manned Spacecraft Center, because the Apollo program is still in progress, and will be for some time, thus making it desirable to examine all the documents at this single location.

Consideration was given to the feasible methods of duplicating the documentation. The alternatives are

Microfilm,
Microfiche,
Paper copies,
Aperture cards, or
Computer tapes.

From the point of view of the initial cost, microfilm and microfiche are slightly more expensive than the hard copies. This is borne out by the Boeing Co. quotations for copying the Lunar Orbiter documents. The cost of supplying paper copies is approximately $3600 less than film copies. However, film copies are better suited to automated search and retrieval from the total inventory collection of documentation. If the documentation is stored on microfilm at the outset, considerable effort may be saved later in the event that in-depth searches must be made.

Many of the documents now stored are in the form of aperture cards. Aperture cards, while basically yielding the same information as the films and hard copies, possess an advantage over the other forms with the exception of computer tape, in that they are easily retrieved using a machine sorter. The principal drawback is the fact that the film size constraints require two cards for many drawings for complete coverage.

Storage of the documentation in the memory bank of a computer must be considered, since techniques exist for the reproduction of drawings, circuit diagrams and similar representations. The computer has not been considered as the prime storage and retrieval system for the Ranger, Orbiter and Surveyor, because the expense of conversion to this type of system is unwarranted at this time. If it were planned to convert the documentation of these missions to a form usable on a computer, considerable time would have to be devoted to programming and input. This goes beyond the requirements of the Space Science Board, and should not be done without certain knowledge that there will be sufficient demands on the system to justify additional expenditures.

C. Other Alternatives for Documentation Preservation

In the event that the demands from the investigators are sufficiently numerous, and require more information than is available under the intended inventory collection plan, it will become necessary to augment the system to enable retrieval of more detailed materials information. Therefore requests were made of the contractors for estimates of the cost of extracting the materials data and transferring it to NASA in a usable form. The costs indicated here reflect the fact that the selection would be done by personnel experienced with the projects, resulting in cost savings due to time lost by inexperienced personnel in learning about the projects.

The Hughes Aircraft Co. proposed that the following six tasks would be required to accomplish this selection for Group A and Group B Surveyor spacecraft.

1. Obtain a list of materials approved for Surveyor and identify those containing organic material.
2. Review Indentured Parts and Drawing Lists, for Groups A and B configured spacecraft, and identify those drawings potentially containing organic material.
3. Obtain Duplicate Aperture Cards (DACs) for each drawing. On the average, two (2) Duplicate Aperture Cards are required per drawing, since the area covered by a DAC "frame" is limited to 44 inches.
4. Review the DACs for organic material and identify the type and amount of material involved.
5. Compile a matrix of the amount of organic material by type and subsystem or control item.
6. Conduct a study to determine the amount of organic materials contained in the rocket engine products of combustion, which would remain on the lunar surface.

It is estimated that the above effort would require 17 man-months and $1500.00 in Other Direct Costs (materials and reproduction). The

estimated total fixed price for this job, including general and administrative expense and profit, is $53,300.

In the case of the Lunar Orbiter Spacecraft, the Boeing Co. personnel who are intimately familiar with the spacecraft are still available and could accomplish the following tasks:

1. Examine all drawings and documentation.
2. Determine the composition of the airborne hardware.
3. Furnish a report containing the classification of the organics and their approximate weight.

This effort would require six months of effort at a cost to the Government of $105,591.

Exotech has been unable to get a firm quote from JPL with respect to the Ranger documentation costs for similar efforts.

AIMP documentation is to a large extent already broken down in the manner described and little additional effort is required to maintain the documentation in this form.

The estimated cost of completing the materials documentation for the Apollo program, copying it into a suitable format and entering it into the current COMAT/TRIS System is $50-100,000. It should be noted that many thousands of documents exist in the hands of approximately 600 contractors, sub-contractors and other suppliers. The content of these documents in terms of organic materials is unknown at this time, and this uncertainty contributes significantly to the cost spread.

D. Dispersion Models

A survey of analytical methods for evaluating the dispersion of contaminants from hard impacts and soft landings was conducted during the period of this effort. This survey was carried out in order to determine the applicability of existing models to the determination of contamination risk areas, and the requirements for programming the models into the Interactive Computer Information System for Planetary Quarantine for Lunar

Programs. Two models[5,6] were found suitable for use in the predicting contaminant spread as a result of landings on the Moon's surface.

1. Soft Landings

The model by Aronowitz et al covers the chemical contamination of the lunar surface by LM exhaust during a soft landing. The total contaminant distribution is bifurcated into two phases of contamination: a far field distribution and a near field distribution.

a. Far Field Distribution

The gas plume issuing from the LM descent rocket engine nozzle into the vacuum around the moon interacts with the lunar surface causing contamination of the surface. The rocket plume has two major flow regimes. Adjacent to the nozzle exit there is a compressible continuum fluid flow regime, but as the gas continues to expand out from the nozzle the density decreases, and a free molecular flow, far field regime develops.

When the LM vehicle, in its landing trajectory, is at an appreciable altitude, only the fully developed far field of the exhaust plume intersects the moon. This interaction produces the far field contamination that has been analyzed and determined by assuming free-molecular point-source flow of the exhaust gas in the lunar gravitational force field.

Flow Model - The flow model thus consists of a moving, free molecular-flow point source in the lunar gravitational force field. The velocity of the gas

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[5] Grumman Research Dept. Report RE-242 - Investigation of Lunar Surface Chemical Contamination by LM Descent Engine and Associated Equipment by L. Aronowitz et al.,March 1966.

[6] Report SC-M-68-539 "The Chances of Retrieval of Viable Microorganisms Deposited on the Moon by Unmanned Lunar Probes", by Martin S. Tierney, Sandia Laboratories, Aug. 1968.

molecules flowing from the source is the vector sum of the velocity at which the source (LM) is moving and the source exhaust velocity. At ignition of the descent engine the LM velocity is approximately half the exhaust velocity and so must be included in the analysis. The random thermal velocity is considerably smaller. The molecules follow orbital trajectory flight paths that may intersect the spherical lunar surface where, as a first approximation, they can be assumed to be fully adsorbed.

Analysis for Contamination Calculation - The total far field contamination distribution on the lunar surface is obtained by integrating at each of a series of fixed lunar points the time history of contamination flux for the time period of the far field portion of the LM landing trajectory. The input data (LM position and velocity and the point source exhaust velocity and density factor distribution) are such that the integration must be done numerically by determining the flux at discrete times over the powered descent phase of the LM trajectory.

The principal equation in the flux calculation is the standard gravitational-force-field particle-trajectory equation that defines the flight path of a particle as a conic section. This equation is most easily solved in a spherical coordinate system with origin at the center of the Moon and with polar axis going through a known point on the trajectory.

To calculate the total contamination at a fixed point on the lunar surface, the particle trajectory equation must be applied repeatedly to the source

as it moves along the LM trajectory. The movement of the source means that the local coordinate system for the particle trajectories rotates relative to the fixed point. Furthermore, the particle trajectory equation does not explicitly determine which particle will land at the fixed point. To circumvent these difficulties, a different, indirect approach must be taken. Therefore, at a given time or, equivalent, for a given position of the source, the velocity that a particle must have at the source to intersect the fixed point is calculated and this velocity uniquely determines the particle flux at that point.

b. Near Field Distribution

The near field distribution is concerned with the study of lunar contamination by the LM rocket gases when the vehicle is close enough to the Moon such that a region of continuum fluid mechanics exists from the exhaust nozzle down to the lunar surface. This problem is considered as the near field erosion problem. For purposes of this study, erosion characteristics will not be considered in the determination of contamination. However, the program can be used to calculate the redeposited particle distribution on the surface and the associated temperature testing for a suspension model. A conclusion reached in the near field distribution research is that particles of 0.1 mm radius may fall as far as 130 meters from the rocket nozzle centerline.

c. Adsorption Estimation

Adsorption of Rocket Exhaust Gas on the Lunar Surface has been calculated using a solid lunar surface

model. Adsorption of the LM descent rocket exhaust gas on lunar surface material can introduce significant amounts of contaminants into the samples of the lunar surface that the Apollo astronauts will bring back to earth for scientific analysis. Discussed herein is a model used for quantitative calculations of the amount of rocket gas adsorbed on the lunar surface, and the subsequent desorption of these surface contaminants.

The model chosen for the lunar surface is a rough plane. This choice agrees well with the current knowledge of the lunar surface. The composition of the lunar surface material, in this model, was considered to be mainly metal silicates.

As the LM descends toward the touchdown site, gas molecules from the rocket exhaust will strike the lunar surface. While the LM altitude is above 100 or 200 feet, the molecules striking the surface are in the free molecular flow regime. At lower altitudes, the gas contacting the lunar surface in the vicinity of the LM is in the continuum flow regime. The formulation uses gas-dynamic equations appropriate to the continuum regime.

d. Computer Program Usability

The computer programs developed for the contamination distribution estimates for both Far and Near field distribution as well as the adsorption computations are operational on an IBM 7094 digital computer. It is reasonable to conclude that these programs have been written in FORTRAN, a widely used scientific computation language. It is felt

that with some modifications to these programs, conversion to the CDC 3100 may be possible.

2. Hard Landings

The Sandia report[7] describes the dispersonal of contaminants during hard landings. Two possibilities are modeled.

- Dispersion of lunar soil ejected by a spacecraft making a hard landing on the Moon.
- A range distribution for fragments of a spacecraft making a hard landing on the Moon.

The range distribution model can be used to determine the probability that beyond a given distance from impact point no fragmentation is expected to be found.

The assumptions under which the range distribution probabilities are derived should be noted.

- Impact of the lunar probe is normal to the Moon's surface.
- Fragments are ejected isotropically.
- Angle of ejection of a fragment is independent of the fragment mass and speed.

The soil ejection model was not used. Its output is given in surface density of crater ejecta per square kilometer rather than fragmentation distribution.

The interaction aspects of the Planetary Quarantine Lunar Programs Information System are not operational, however, the computational algorithms for the hard impact model are operational on the CDC 3100 computer. The soft landing model is operational on an IBM 7094 computer.

3. Applications of Dispersion Models

Table 3 gives a listing of the latest information available on landing sites, impact mass, velocity, and date of contact. The location of Orbiter IV, previously unreported, was

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[7] Tierney, Loc.Cit.

TABLE 3
SPACECRAFT LANDING COORDINATES

U.S	LAT.	LONG.	AREA NAME/CRATER	IMPACT MASS/KG	SPEED KM/SEC	DATE OF CONTACT
Ranger 4	13.9°S	129.4°W	*	331	2.669	4/26/62
Ranger 6	9.3°N	21.4°E	Mare Tranquillitatis	365	2.66	2/2/64
Ranger 7	10.7°S	20.7°W	Mare Cognitum	366	2.616	7/31/64
Ranger 8	2.7195°N	24.6195°E	Mare Tranquillitatis	367	2.651	2/20/65
Ranger 9	12.9°S	2.4°W	Alphonsus	366	2.669	3/24/65
Surveyor 1	2.46°S	43.32°W	Oceanus Procellarum Flamsteed	270	3.96 m/sec	6/2/66
Surveyor 2	4±0.4°N	11±1.1°W	Sinus Aestuum	292	2.38 m/sec(?) crash	9/22/66
Surveyor 3	2.99-3.06°S	23.32-23.34°W	Oceanus Procellarum	285	Soft Landing	4/19/67
Surveyor 5	1.45°N	22.25°E	Mare Tranquillitatis	281	Soft Landing	9/11/67
Surveyor 6	0.46-0.51°N	1.37-1.39°W	Sinus Medii	282	Soft Landing	11/9/67
Surveyor 7	40.89°S	11.44°W	Tycho	284	Soft Landing	1/10/68
Surveyor 4	0.42°N	1.33°W	Sinus Medii	284	U	7/16/67
Orbiter 1	16.35°N	160.71°E	*	387	2.38	10/29/66
Orbiter 2	3.0°N	119.1°E	*	392	2.38	10/11/67
Orbiter 3	14.6°N	91.7°W	Einstein	387	2.38	10/9/67
Orbiter 4	0±10°	26±5°W	Lansberg	392	2.38	10/6/67
Orbiter 5	2.79°S	83.04°W	D'Alembert Mts. Schluter	392	2.38	1/31/68
Apollo 10 DS						
Apollo 11	0°41'15"N	23°26'E	Mare Tranquillitatis West Crater			7/20/69
Apollo 12	3°S	23.3°E	Oceanus Procellarum			
Apollo 13	6°S	17°W	Fra Mauro			
Apollo 14	0°36'S	32°43'E	Censorinus			
Apollo 15	22°12'N	29°20'E	Littrow			
Apollo 16	41°45'S	11°30'W	Tycho			
Apollo 17	13°45'N	56°W	Marius Hills			

(cont'd)

SPACECRAFT LANDING COORDINATES

U.S.	LAT.	LONG.	AREA NAME/CRATER	IMPACT MASS/KG	SPEED KM/SEC	DATE OF CONTACT
Apollo 18	25°9'N	49°30'W	Schröter's Valley			
Apollo 19	8°3'N	6°E	Hyginus			
USSR						
Luna 2	30.1°N	0.01°E	Mare Imbrium/Autolycus	390	2.38	9/14/59
Luna 5	1.5°S	25°W	Oceanus Procellarum Lansberg	1476	2.38	5/12/65
Luna 7	9.8°N	48.8°W	Oceanus Procellarum/Marius	1506	2.38	10/8/65
Luna 8	9.6°N	61.6°W	Oceanus Procellarum Galilei	1552	2.38	12/6/65
Luna 9	7.8°N	65°W	Oceanus Procellarum	1360	5.5-6.1 m/sec	2/3/66
Luna 13	18.5°N	62°W	Oceanus Procellarum	100	Soft Landing	12/24/66
Luna 15	U	U	Mare Crisium	U	1.3 Km/sec (?)	7/21/69

* Not named
U Unknown

supplied by the Data and Tracking Group at JPL. Luna 15 data are not listed, due to the fact that at the time of this report, Exotech has been unable to acquire any information other than the report made by the Jodrell Bank Observatory during the Apollo 11 flight.

The proposed dispersion models must be combined and programmed with terminal trajectory information and impact sites to answer queries such as these

- What is the probability of the presence of a fragment or fragments of a specified size or mass at a designated sampling site?
- Which mission(s) would be the principal contributor(s) to contamination in a particular lunar region?
- At given distances from the impacts, what size range of fragments is to be expected?
- What is the closest point of approach to a previously landed spacecraft where the probability of detectable contamination is less than a selected value?

In order to provide answers to these and similar questions, both models must be utilized in such a way as to maintain their separate identity, and allow their outputs to be supplementary or independent. In this manner, the exhaust components from soft landers and fragments from impacts can be reported as part of a total contamination picture or as separate constituents.

E. Scope of Materials Documentation

The analytical efforts of the Lunar Principal Investigators are frequently directed toward the identification of trace amounts of components. If the analysis reveals the presence of a substance such as sulfur or phosphorus, the investigator will be concerned as to whether it is of terrestrial or extraterrestrial origin. The investigator will have an

interference problem whether the sulfur comes from sodium sulfate or from a type of rubber that deteriorated on the lunar surface. After prolonged periods of time, it may be that the two materials are virtually indistinguishable from each other. It would be helpful to the investigator, in any case, to have an estimate of the probability that a certain amount of sulfur can be expected as a contaminant in the area of interest.

From the point of view of the life scientists, in contrast to that of the analytical chemist, noted above, the source of the contaminant is important. After some treatments, notably gas chromatography/mass spectrometry, the original composition is destroyed, and the inorganic ions are the same, regardless of the source. The phosphorous from a phospholipid is indistinguishable from that from a phosphate, after treatment nitrate nitrogen appears the same as that from an amine. With additional effort, differences can be identified, but the problem is obvious.

The information available from the inventory would be more comprehensive if the scope were broadened to include all non-metallic materials, especially those materials which include their composition elements of biological composition. This list should include those elements such as N, P, S, Na, K, Mg, Ca, Sr, F, Cl, Br, and I for example and any others which have been found necessary for terrestrial life and are present in non-metallic compounds.

F. Future Requirements

The compilation of a lunar inventory of possible surface contaminants is predicated upon the fact that future needs will require information concerning the identity of these materials. It is obvious that the spacecraft documentation preserved in its present status cannot answer these projected needs. An assessment of future requirements is needed, since any detailed characterization, indexing or categorization of the documentation beyond that of identification according to missions is unwarranted (except for Apollo) at this time unless a real demand is expected.

A program should be initiated which will enable the prediction of these needs and thereby permit planning to accommodate them. Certain assumptions have been made in this study concerning future needs, although

the information needed for valid estimates is not available at this time. The first assumption that must be considered is whether or not justification exists for expecting that this documentation will be requested. The rationale for saying that it will be requested is based upon the expectation that the next generation of analytical instrumentation will operate at increased sensitivity levels, i.e., where parts per billion sensitivities are now commonplace, parts per trillion are likely to be obtainable in the near future. If this is indeed the case, background levels will have to be examined very carefully, and this may lead to requests for more details on the materials which make up this background.

Along the same line of thought, a twenty year period recommended for retention of documentation is considered sufficient to cover any future need for the inventory.

The information requests received during this period will also establish whether or not more detailed procedures are needed for a storage and retrieval system to manage the information contained in the inventory.

G. Maintaining the Inventory

During the study, considerable difficulty was experienced in attempting to update the landing parameters and location coordinates for several of the missions. In order to maintain the inventory with the best available information, such data should be frequently reviewed and updated. At present, they are scattered among the agencies responsible for the programs, and become less reliable as the groups change. A central source is needed which can provide this type of information to interested personnel. Since much of this effort is directly related to the manned spacecraft program, MSC, Houston is the logical Center to set up and maintain all data on landing sites, impact characteristics, and other pertinent information on lunar mission hardware. An effort should continue to include all available information on USSR landers as well.

In describing the potential usefulness of the inventory, an incident of recent occurrence should be noted. No plans have been made to check the accuracy of the model formulated by Aronowitz for soft landers either

by analyzing specimens taken near the LM on Apollo 11 and 12, or those taken from the crater containing Surveyor 3. A recent publication describing the LM exhaust products has been published[7], and has apparently enabled one researcher to identify a fluorescent material as having originated in the LM exhaust. Considerable benefit would result from verification of this model enabling the prediction of uncontaminated sampling sites, and in the degree of contamination to be expected close to the LM.

The only discrimination of classes of materials presently available in the inventory documentation is that of the Apollo program which separates out the non-metallic components. This is the only limitation applied to the collection of materials information and is a more realistic distinction than organic/inorganic. By increasing the prescribed scope of the inventory documentation to this extent, possibilities of omission would be decreased considerably at little cost since the Apollo documentation system is already observing this division. If this concept is adopted, the inventory would be capable of providing more comprehensive information than would be possible with an arbitrary elimination of inorganic materials.

Additional useful information which could be entered into the Planetary Quarantine computed-based information system includes the locations of the various sub-sections within the spacecraft documentation and general cross-reference index. To illustrate: Although the spacecraft materials information will not be analyzed or categorized, the primary index titles as utilized by the various contractors in filing the information could be carried through. Requests for information relating to a particular subassembly on Ranger VII could also provide file locations on similar subassemblies on Rangers VIII, IX, IV and VI at the same time. If information concerning the desired mission is incomplete similar data for an identical spacecraft could be indicated.

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[8] Apollo Lunar Module Engine Exhaust Products, B.R. Simoneit, A.L. Burlingame, D.A. Flory and I.D. Smith, Science, Vol., 166, No. 3906, Nov. 7, 1969

IV. RECOMMENDATIONS

It is convenient and useful to present recommendations separately for (1) the preservation of documents and, (2) the implementation of a materials information system. The first category deals largely with detailed data on types and quantities of materials contained on particular flight vehicles for which there may or may not be a demand. The second category represents the general methods for providing to potential users information on the risk or likelihood of sample contamination and, if appropriate, also on the type, quantity and source of the contamination. The documentation category is therefore a subelement of the information system. However, since action taken on the preservation of documents - and more significantly, the lack of such action has an irreversible effect on the future usefulness of an information system, the recommendations concerning the preservation of documents require early attention.

A. Implementation of an Information System

To assure that costs are compatible with known benefits, it is recommended that implementation of an information system be carried out in two steps as follows:

1. Preparation of Contamination Risk Model for the Moon

Information concerning the likelihood of sample contamination by materials of terrestrial origin (due to prior lunar flights) should be prepared and made available to interested personnel within NASA and the scientific community. This is most readily accomplished either in tabular form or as a map of pertinent regions of the Moon containing contamination probability "contours".

The updating of the system at KSC with all pertinent lunar mission spacecraft parameters is required. The information is currently not available from a single source, but must be sought from the various responsible agencies.

The dispersion models of Aronwitz and Tierney should be programmed into the Planetary Quarantine computer system

in a manner that will provide identification of surface areas in the vicinity of spacecraft landing sites where contamination may affect the samples. In order to apply the dispersion models delineated by Aronowitz and Tierney, and to specify their effect on the various sampling sites, the best available information on the impact parameters and locations of the spacecraft must be applied. This input to the inventory should be updated as better information becomes available. It should be noted that the inclusion of this input to the information system is important in order to provide estimates of the distribution of contaminants over the lunar surface, and to provide an estimate of the probability of a contaminated surface sample being drawn from a particular region of the Moon. The more precise these inputs are, the better the estimates will be. Unlike the materials documentation input which treats U.S. spacecraft alone, this information treats all spacecraft, including those originating in the USSR.

The most important output from this system, initially, is the delineation of the contamination risk areas. The intended Apollo landing sites through Apollo 20 have been tentatively selected. Issuance of the expected contamination levels (risk areas) in these and adjacent areas would be valuable aids in planning these missions.

2. Assessment of Future Requirements

With the information now available, the precise questions to be expected from investigators of lunar sample materials cannot be predicted at this time with sufficient certainty. Provisions for gaining additional information on potential requirements are therefore necessary.

It is recommended that a questionnaire be distributed to recipients of the Lunar Contamination Model. The questionnaire should determine the aim of the research, the general

techniques and instrumentation to be used, the elements of interest, the interferences, the expected use of the data system, particular types of information desired, and other relevant data. From these questionnaires, estimated usages can be made, conclusions as to indexing requirements can be drawn and the type of retrieval system which will be most useful can be selected.

B. Preservation of Documents

1. Minimum Requirements

The fundamental consideration with respect to spacecraft materials documentation is that disposal or loss of pertinent records be prevented so long as a possible need exists for such information to support lunar sample investigations. The mechanism for preservation of these documents exists in the retention time requirements imposed upon the various contractors. This should be supplemented by a requirement for notification of the Planetary Quarantine Office prior to disposal or transfer of the documents from their present locations. Implementation of a notification requirement would ensure against inadvertent loss of the information.

2. Recommended Storage Procedures

A more direct approach to the solution of the problem is to order transferral of the documentation, or suitable copies thereof (see below), to the Federal Records Center nearest the cognizant agency at the end of the contractually specified retention time for technical data. The exception is the Apollo documentation In this area, the COMAT/TRIS system should be expanded to contain the additional documentation required for all nonhabitat areas, e.g., the descent stage. The documentation need not be completely detailed with respect to test results of flammability or tests of other types pertinent to the current needs of the

system, but should contain all nonmetallic organic materials information pertaining to amounts, classifications, and specifications of the material. Incoming documentation should be separated into two categories: (1) that which contains no materials information, (2) the remainder. Category (2) should be indexed with a brief descriptive title and entered into the system. The responsibility for locating and transferring the documentation should be shared by the personnel currently managing the system, aided by the prime contractors, North American Rockwell, Grumman Aircraft and the Manned Spacecraft Center. The urgency of this move cannot be overstressed since construction of the remaining Apollo spacecraft is now virtually complete and personnel now assigned to these projects are likely to be transferred. With this action, the location of many documents in the hands of suppliers will become obscured. The documents pertaining to the habitability areas of the CM and LM are necessary, but in themselves not sufficient for the inventory, because the descent stage of Apollo 10 will impact at a future date, and the ascent stage from Apollo 12 impacted on November 20, 1969.

3. Suitable Copy Recommendations

Of the formats described previously, the one that is most suitable for both present and evisioned future needs is the aperture card. It is readily indexed in any way desired, lends itself to rapid and selective retrieval, requires little storage space in relation to the amount of information it can carry and is durable enough to withstand a great deal of handling. It is therefore recommended that the aperture card be established as the standard copy for all information to be copied for transferral, and that this also be the form that any future indexing or categorization should follow.

A requirement should be issued that the documentation concerning nonmetallic materials, their specifications, and other pertinent data, but excluding such items as flammability tests, for all future lunar missions be sent to the COMAT system for inclusion into the inventory. This will aid in keeping future search costs down. It is likely that little further documentation can be expected under the Apollo requirements except for the Lunar Vehicle, but this will constitute a large amount of material input that will not have to be processed at a later date. This can also accommodate the inputs from the possible lunar orbit shuttle vehicles for extended exploration of the surface.

In the event that requests from principal investigators warrant the additional effort necessary to provide more specific information concerning the organic materials, it is recommended that the COMAT/TRIS system format be used as a nucleus for an active inventory system.