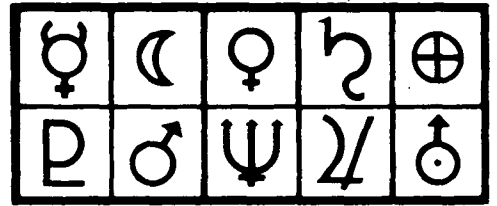


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PLANETARY QUARANTINE

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August 1972

FINAL REPORT OF PLANETARY QUARANTINE ACTIVITIES

Prepared by the
Planetary Quarantine Department

SANDIA LABORATORIES



OPERATED FOR THE UNITED STATES ATOMIC ENERGY COMMISSION BY SANDIA CORPORATION | ALBUQUERQUE, NEW MEXICO, LIVERMORE, CALIFORNIA

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Prepared by the
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Abstract

This report summarizes the activities of the Planetary Quarantine Department at Sandia Laboratories during the period April 1965 through June 1972. Included are the rationale, the methods and the results of modeling and experimentation used in dry heat, radiation, thermoradiation and chemical sterilization studies. Publications describing these activities and accounts of closely related research are also furnished.

Most of the work described herein was conducted under the following
NASA contracts:

W-12324, Office of Technology Utilization

R-09-019-040, Bioscience Division, Office of Space
Science and Applications

H-13245A, George C. Marshall Space Flight Center

W-12853, Planetary Programs, Office of Space Science
and Applications

In addition, some part of this work was supported by the United States
Atomic Energy Commission.

Foreword

This final report summarizes work done for the NASA by the Sandia Laboratories, a prime contractor to the AEC, under the terms of AEC-NASA Memorandum of Understanding AT(29-2)-1816. Assistance was provided in support of the NASA Planetary Quarantine Program during the period April 16, 1965 to present. The cognizant NASA official during this period was Dr. Lawrence B. Hall, Planetary Quarantine Officer. His support and encouragement contributed in a major way to the effectiveness of the activity and they are greatly appreciated. It is hoped that the contributions by Sandia Laboratories will continue to prove beneficial to the NASA in their work toward a successful planetary exploration program.

The report covers the following major areas of activity: development of the motivation for the work, the methodology employed, the results obtained and the interpretation of the results. Although all the information may not be covered in each project description, the supporting documents which develop the detail are listed at the end of each topic discussed. Information concerning the availability of these documents may be obtained from the Biological Sciences Communication Project, The George Washington University Medical Center, 2001 S Street, N. W., Washington, D. C., 20009.

Sandia Laboratories involvement with the national planetary quarantine community has resulted in personal associations which have enriched the skills and experience of our personnel and aided in the successful completion of many of the activities described herein. Our thanks are due these many persons, and to the extent that they become less frequent, these associations will be missed.



H. D. Sivinski, Manager
Planetary Quarantine Department

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Department Quarterly Reports are listed as Items 1-23 in the Publications Section, with the item number also being the quarterly report number. Other publications are listed as Items 24-127.

Under Subtopics and References, at the end of each major subsection, Quarterly Reports are referenced by item number and page number; other publications are referenced by item number.

Section I

General Studies

This section contains a description of some of the general studies, both experimental and theoretical, undertaken in support of planetary quarantine that do not fit naturally into the specific areas of activity presented in subsequent sections.

I.A

Problem Definitions

One of our initial activities in planetary quarantine was aimed at finding realistic ways of deriving non-contamination requirements for specific planetary missions in the face of considerable uncertainty about:

- The precise nature of what "contaminating" a planet means,
- The technology base available for a posteriori assessment of contamination probabilities,
- The time period over which any planet might be quarantined,
- The number and types of missions to any planet during its quarantine period.

We first demonstrated that the model proposed by Sagan and Coleman for deriving mission non-contamination requirements was not conservative because mission requirements derived from this model became more strenuous as the number of missions became finite.

At about the same time, a very simple model was proposed which required the estimation of an upper bound, U , on the number of missions to be flown to a given planet during its quarantine period. With such an estimate, mission non-contamination requirements could be derived for the number of estimated missions only. The simple character of this model made it intuitively desirable, but its use presented a problem. Most of the aforementioned uncertainties were handled by making U large. But since individual mission requirements derived from this model became more demanding with increasing U , too large a value of U was undesirable. On the other hand, if U was estimated too low, no means was available in the model for subsequently meeting overall planetary quarantine objectives.

To overcome this problem of "balancing" U , a multi-stage decision model was developed which permitted periodic estimates of the number of missions (of various types) and then specified subsequent mission requirements. The model had the following properties:

- When first used, (stage 1) it had precisely the same form as the simple model above,
- Estimates of the numbers of missions could be made realistically, and penalties for misestimating could be analyzed a priori,
- In the event of a misestimate of the number of missions to a planet, new mission requirements could always be derived which would still permit the attainment of overall planetary quarantine objectives for that planet, and
- New information gained about a planet in the course of investigating it could be used to influence future mission requirements.

Parametric analysis of the model identified several sensitive areas in planetary quarantine where conservative approaches were desirable. For example, in estimating probabilities of release and growth, etc., these should be chosen "conservatively" in the sense that great difficulties would be encountered in the future should it ever be necessary to revise these values (even slightly) upward from their initial values. More general conclusions were also drawn from the model analysis. For example, it was concluded that continuing support should be given to the development of better sterilization methods as long as there exists uncertainty in the exploration program and a need for planetary quarantine.

During this same time period, another potential problem associated with achievement of planetary quarantine objectives lay with the then accepted and rather vague definition of what constituted "contamination of" a planet. Specifically, the definition at that time implied a need to estimate "the probability that a given microorganism landed on the surface of Mars will multiply and contaminate a sizeable fraction of the planet." We proposed that one might more rigorously analyze the contamination of a planet if contamination were taken to mean the deposition of sufficiently many life forms (which might be one or more) to "bias any further biological experimentation" on the planet. A rigorous analysis of this approach, including accounting for different modes of release, was published.

Once a fairly rigorous definition of contamination was developed, and individual mission non-contamination requirements could be derived, the next logical class of problems to be addressed was the identification of all factors which would have a bearing on the achievement of mission requirements. This was done in "tree" form, with a hierarchical description of objectives in which top level objectives corresponded to

mission requirements and lower level objectives corresponded to actions that were possible and also necessary to meet those objectives at higher levels. In this way, five general classes of problem areas, and specific problems in need of resolution in each area, were identified. These findings were presented to the Planetary Quarantine Advisory Committee (now Panel) in mid 1968. Among those problems identified were:

- A need for better understanding of dry heat sterilization,
- A need for better sterilization techniques,
- A need for better understanding of the sterilizing effects of radiation in space,
- A need for better models for bioburden estimation and prediction (particularly since this impacts sampling requirements),
- A need for a thorough analysis of what constitutes "contamination of" a planet,
- A need to more fully consider planetary quarantine constraints in spacecraft design.

These problem areas greatly influenced our activities, as can be seen from the titles of subsequent sections in this report.

Subtopics and References

- Sagan - Coleman Model Analysis; 1:7, 2:6, 3:3, 4:4, 85, 93, 95
- Multi-Stage Decision Model; 5:5, 6:3, 93, 95
- Definition of Contamination; 85
- Release of Organisms from Spacecraft; 85
- Conservatism in Planetary Quarantine; 93, 95
- Objective Hierarchy; 4:3, 5:3, 6:3, 96, 99

I.B

Consulting

During the past six years, a number of activities undertaken were of an isolated, one-time nature, generally requested by others in the planetary quarantine community. Many of these activities were either only peripherally related to, or an overview of, the major categories of activity summarized in this report. The following is a list and brief description of some of these "consulting" activities:

1. The extension of the JPL assembly contamination model from a deterministic model to a stochastic one.
2. The undertaking of a management systems study for the Planetary Quarantine Office covering:
 - The nature of administrative objectives and responsibilities,
 - Documentation and information needed to achieve these objectives and meet these responsibilities,
 - From whom and to whom such documentation and information must flow,
 - Effects of management "style" on information flow, and organizational characteristics.

A self-sufficient, program independent, quality control/assurance role and "management by exception" style was recommended for the Planetary Quarantine Office. This study led to our participation in early draft preparation activities of NASA project and contractor requirement documents.

3. The participation on Planetary Quarantine Advisory Committee (now Panel) Subcommittees, specifically, The Mathematical Modeling Subcommittee, and The Subcommittee to Review the JPL Bioburden Model. The latter activity apparently resulted in at least some improvement in bioburden estimation and prediction systems.

4. The review of an Ames document (a draft) advocating the need for no planetary quarantine precautions on Jovian missions which enter Jupiter's atmosphere at or about escape velocity. The work did not support such a strong conclusion.
5. The preparation of reports to COSPAR. For example, in the last United States Space Science Program Report to COSPAR (15th Meeting), the work reported under "Sterilization and Quarantine" was done exclusively at Sandia.
6. A concern for Technical Utilization Activities. This area includes such topics as publicization of thermoradiation, the vacuum probe, and clean room technology in sectors other than those for which they were developed. This has been accomplished by sponsorship of symposia, special sessions at technical meetings, cooperation in preparing Technical Utilization sponsored publications and many personal contacts with potential users.
7. The estimation of the typical amount of radiation absorbed by a Martian lander mission. This study showed that little radiation (approximately 2000-4000 rads, depending upon conditions) is seen by a typical shielded Martian lander. The bulk of this radiation comes from the radioisotopic thermal generators on board.
8. The investigation of the frequency of occurrence on a spacecraft of those organisms most likely to grow on Mars, viz., anaerobic psychrophilic organisms. Some spores are known to belong to this class of organisms, and if they might be found on spacecraft, their hardiness becomes a serious factor in planning sterilization cycles. Our preliminary results indicated that anaerobic psychrophiles are present in Cape Kennedy soil and represent 5% of the total bacterial population.

Subtopics and References

Assembly Contamination Model; 1:8, 84

Management Systems Study; 125

COSPAR, United States Space Science Program Report to COSPAR, 13th, 14th, 15th Meetings, 1970, 1971, 1972, respectively.

Martian Lander Radiation Burden; 18:50, 45

Section II

Dry Heat Studies

This section briefly describes our activities in the area of dry heat sterilization. The need for a better understanding of this phenomenon, with the attendant implication of higher confidence in sterilization cycles and the possibility of designing less severe cycles, was recognized in earlier general studies described in the previous section.

II.A

Problems In Dry Heat Sterilization of Spacecraft

From the outset it has been clear that spacecraft sterilization by dry heat presents some rather unique problems. The two major problems may be stated as follows:

1. A posteriori sampling for sterility is not possible. Therefore a means of predicting sterility with high confidence is needed.
2. Because of the complexity and desired high reliability of a spacecraft, sterilization cycles should be of the least possible severity.

Problem (1) implies the need for a model in which one has a great deal of confidence. To motivate the activities described below, the general state of "death" modeling a few years back must be appreciated. The model most in favor at that time was the so-called "logarithmic" model in which the expected number of survivors $E(n(t))$ at time t is given by the equation

$$E(n(t)) = n(o) 10^{-t/D} \tag{II. 1}$$

where D is an empirical parameter determinable from survivor data - but having no further meaning. Data which deviate from the type of behavior described by Equation II. 1 have been reported since the early 1900's and are still being reported.

Anomalous behavior has been variously ascribed to clumping, moisture, germination, and "inherent" heterogeneity. A series of experiments was performed in which it was demonstrated that there are times when none of these "explanations" for non-logarithmic behavior are valid - save possibly the last, by definition. In view of all this, one could have little confidence in predicting sterility using Equation II. 1 unless a posteriori sampling for sterility and, possibly, extra severe sterilization cycles are permitted. These conditions for the confident use of Equation II. 1 conflict with the spacecraft sterilization situation described above. Briefly, then, it was our view (and still is) that spacecraft sterilization should not be handled as a classical sterilization problem. In particular, it was our belief that a model based on more fundamental knowledge was required to describe survival. Such a model

should be capable of agreeing with the various forms of survival data for "homogeneous" populations and lend insight into both the behavior of naturally occurring populations and the influence that other environmental factors will have on sterilization cycles.

Subtopics and References

Dry Heat Sterilization Problems; 2:7, 5:9, 6:5, 7:3, 8:3, 9:3, 27, 28, 31, 36

Rationality; 5:9, 6:5, 7:3, 8:3, 9:3, 28, 31

Experiments vis-a-vis Non-Logarithmic Survival; 9:3, 10:14

II. B

Modeling and Rationale

The objective of the dry heat survival studies was, thus, the development of a rational model for the prediction of microbial survival as a function of sterilizing environment. The environmental parameters of particular interest were temperature, relative humidity, and pressure.

A rational model was desired because of the necessity for confident extrapolation outside the range of feasible observation for establishing sterilization cycles for the then anticipated spacecraft bioburdens and because of the implied greater confidence in predictions. In final form, the model was based on Eyring kinetics under the assumptions that:

1. Microorganisms are sterilized independently.
2. In the environment of interest, sterilization is the consequence of chemical reactions.
3. These reactions have order.
4. There may be competitive mechanisms for sterilization.

More specifically, it was postulated that several types of reactions may be taking place. Schematically,

- | | |
|---|-------------|
| i) $A \rightleftharpoons B \rightarrow X$ | (1st order) |
| ii) $A \rightarrow Y$ | (1st order) |
| iii) $2C \rightarrow Z$ | (2nd order) |

with n_1 molecules of A permitted, and (i) and (ii) independent reaction types competing with (iii) in which n_2 of n_3 molecules of C must react for sterility. Each arrow has an associated rate constant.

Under Eyring kinetics, each reaction rate constant k_r is given by an expression of the form

$$k_r = \frac{kT}{h} \exp (-\Delta F^\ddagger / RT) \quad (\text{II. 2})$$

where k is Boltzmann's constant, h is Planck's constant, T is temperature in degrees Kelvin, and R is the gas constant. $\Delta F^\ddagger$ is the energy of activation of the reaction and is given by

$$\Delta F^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger + p\Delta V^\ddagger$$

where $\Delta H^\ddagger$, $\Delta S^\ddagger$, and $\Delta V^\ddagger$ are the enthalpy, entropy, and volume of activation respectively. The symbol p denotes pressure.

A series of laboratory-model experiments which was carried out challenged the validity of the kinetic model. It was established that:

1. The model was capable of agreement with logarithmic, convex, concave, and biphasic data.
2. The model accurately predicted survivors at temperatures different from those used for parameter value determination for logarithmic, convex, concave, and biphasic data.
3. Predictions of survivors when the sterilization temperature is not constant was consistent with observations.
4. Model parameter values for the various $\Delta S^\ddagger$'s and $\Delta H^\ddagger$'s for various kinds of homogeneous bacterial populations are consistent with known values for chemical reactions involving biomolecules.
5. The basic assumptions inherent in the model appeared to be consistent with known behavior of bacterial cells at a molecular level.

Thus, it was felt that, for that time, this kinetic model offered a better way of viewing dry heat survival in spacecraft sterilization than did Equation II. 1 of the previous subsection.

Subtopics and References

Modeling; 4:7, 5:9, 6:5, 7:3, 8:3, 28, 35, 36

Model Challenging Experiments; 6:7, 9:3, 10:14, 11:14, 13:10, 31, 119

Rationality; 2:7, 5:9, 6:5, 7:3, 8:3, 9:3, 12:14, 27, 28, 31, 36

II.C

Environmental Effects

Relative humidity was incorporated as an environmental parameter through the $\Delta S^\ddagger$ terms of the model described in the previous subsection. Equipment was developed for precise control of RH and an extensive series of experiments carried out for incorporation of RH as an environmental parameter in dry heat sterilization. This equipment used the principle of condensing excess moisture from the air at controlled temperature and pressure.

In keeping with the espoused objective of developing understanding with the hope of finding "optimal" sterilization cycles, perhaps the most significant result acquired in studying RH influence on survival stemmed from the following observation: in sorption reactions, $\Delta S^\ddagger$ is a nonlinear function of RH which has a high value at some low RH, becoming smaller on either side of this RH. If this was true for the $\Delta S^\ddagger$'s of the model, then conceivably sterilization would take place appreciable more rapidly at this value of RH than at others in the lower ranges consistent with spacecraft sterilization. This indeed did occur, and the optimal value of RH (at sterilizing temperature) was found to be approximately 0.09%.

Pressure was incorporated as an environmental parameter through the $p\Delta V^\ddagger$ terms. Theoretical analyses and preliminary experimental results agreed within an order of magnitude. Although experimental work on this phase was not fully completed, the preliminary results obtained indicated several things:

1. Theoretically, all other things (including moisture) being equal, decreasing pressure should result in more rapid death of organisms if $\Delta V^\ddagger$ is sufficiently large. The rate of destruction approaches some upper limit, which is independent of $\Delta V^\ddagger$, as p decreases, but the rate of approach depends highly on $\Delta V^\ddagger$.
2. Any accelerated effects in organism death in space will not be evident unless the temperature is above about 45°C.
3. Experimental results at 10^{-5} or 10^{-6} torr will be indistinguishable from results obtained in a space vacuum - from a pressure

point of view. Different moisture behavior at these different pressures may influence results.

4. The assumption that the slow rate of death of encapsulated organisms is due to the increase of pressure with temperature in a closed system leads to values of $\Delta V^\ddagger$ not inconsistent with those anticipated for a very large molecule such as DNA.
5. Values of $\Delta V^\ddagger$ tend to lie in the range between 1 and 100 liters/mole, indicating the possible involvement of a very large molecule such as DNA in the thermal inactivation process. Indeed, the implication is that a "large part" of a large molecule is involved, yielding indirect evidence of DNA denaturation.

From this last, admittedly very indirect, inference, speculation arose about possible synergistic effects between heat and ionizing radiation.

Subtopics and References

Relative Humidity Analysis; 13:6, 14:3, 31, 33

Relative Humidity Experiments; 17:40, 18:53, 19:41, 20:60, 34

Pressure Analysis; 15:3, 16:4, 30, 32

Pressure Experiments; 20:61

II. D

Heterogeneous Populations

As the dry heat resistance of populations of naturally occurring organisms began to be studied, organism strains that apparently were very resistant were encountered. Initial dry heat and thermoradiation experiments (Section IV) using naturally occurring organisms suspended in a 95% ethanol solution clearly indicated that these organisms were more heat resistant than Bacillus subtilis var. niger spores. Further experiments were conducted using these naturally occurring organisms in their natural dry state, i. e., along with the dust particles with which they were originally collected at Cape Kennedy. The results of these experiments again confirmed the high heat resistance of these organisms.

As a further test of the heat resistance of soil organisms, a method was developed to separate naturally occurring organisms from dust particles. Because of its desirable qualities, Freon TF was selected as the dissociation media, and satisfactory yields were obtained from the dust stock. Both Freon TA and Freon TE were also tried for this purpose, although Freon TF produced a higher yield of organisms from the dust. Additional experiments were conducted to determine the effect of preconditioning the dust particles under different relative humidity conditions prior to subjecting them to the separation process. The yield of organisms from dust preconditioned at 20% RH was approximately 3-4 times greater than that from dust preconditioned at 100% RH.

Then, at the request of Dr. L. B. Hall, a series of experiments was conducted to determine whether possible "clumping" of organisms and dust particles might account for the greater heat resistance. Organisms obtained from the Freon separation process were sparsely deposited on petri dishes at a density ranging from 11 to 45 organisms per square foot. After exposure to dry heat, plating, and incubation, these organisms also exhibited high heat resistance and a D_{125C} value of 35 to 40 hours. These experiments showed conclusively that these naturally occurring organisms are highly heat resistant and that the heat resistant property is a natural characteristic rather than the result of protection from clumping with soil particles or other organisms.

Subsequent concern over the existence of these dry heat resistant soil spores at Cape Kennedy prompted us to propose that sterilization plans should be based upon the assumption that the spacecraft's bioburden is a non-homogeneous population with respect to D-values. Through the use of this approach, planetary quarantine objectives could be achieved while using a shorter sterilization time than would be required if the entire population of organisms on the spacecraft was assumed to have a D-value equal to the maximum of any organism on the craft. Indeed, this proposed approach can be shown to be "optimal" in the sense that it genuinely leads to achievement of sterilization goals with the least severe sterilization cycle.

A model based on the heterogeneity assumption described above was introduced. This model, which assumes the log model of dry heat inactivation, is summarized by the equation

$$E(n(t)) = \frac{E(n(0))}{\sqrt{2\pi}\alpha_2} \int_0^\infty e^{-kt} \frac{e^{-(\log k - \alpha_1)^2 / 2\alpha_2^2}}{k} dk \quad (II.3)$$

where

k = the reaction rate associated with the D-value

$n(t)$ = number of organisms remaining after an exposure of
time t to the lethal environment,

$E(n(t))$ = expected value of $n(t)$,

and α_1 and α_2 are parameters related to the mean and standard deviation of reaction rates k within a population and must be determined for the particular population under consideration. Knowledge of these parameters is necessary for computing sterilization times using the above equation. In order to obtain realistic values for α_1 and α_2 for spacecraft sterilization, one must possess information about the survival, in a given lethal environment, of a population of microorganisms having a distribution of resistance similar to that associated with the spacecraft bioburden. Typically, this information is in the form of survival data for a heterogeneous population where t_i , $i = 1, \dots, n$ are the sampling times and y_i , $i = 1, \dots, n$ are the respective mean sample values at these times. Then α_1 and α_2 of Equation II.3 are determined so as to minimize the expression

$$\sum_{i=1}^n [\ln E(n(t_i)) - \ln y_i]^2, \quad (II.4)$$

making Equation II.3 representative of the survival characteristics of the population in question.

The model represented by Equation II.3 is, in a way, a culmination of much past work. To obtain it, the rationality inherent in past thermal death modeling (summarized earlier in this section) was necessary. It was observed that the major variant between "resistant" and "less resistant" spores appeared in the model as a difference in activation entropy, $\Delta S^\ddagger$. This in turn suggested that "stability" of some spore substance(s) was the primary cause of "hardiness". The evidence collected in the past few years that this critical substance(s) was, indeed, DNA led to a physically based model of stability in which $\Delta S^\ddagger$ is log-normally distributed within a random heterogeneous population; and this, finally, yielded Equation II.3.

To use Equation II.3 via Equation II.4 requires heterogeneous population survival data for the determination of the values of the parameters α_1 and α_2 . Two such populations were studied by us: organisms in Cape Kennedy soil and organisms residing in vacuum cleaner dust taken from Building AO at Cape Kennedy. Additional sources of heterogeneous population data were the dry heat resistance studies of Mariner '69 spore isolates reported in the literature.

Using each of these three data bases, the time at 125°C to reach a final spacecraft surface population of 10 organisms was computed (as described above) and compared with the time needed if all spores are assumed to have a 30-minute D-value. This is shown in Figure II.1.

The number 10 in the abscissa label in Figure II.1 is arbitrary and is used only as an example. This axis represents the number of viable microorganisms which are expected to be remaining on the spacecraft surface at the termination of the sterilization cycle. This level is computed as a function of such parameters as probability of growth, probability of survival in ultraviolet radiation, etc. The most significant portions of these curves are on the extreme left. Here we observe that if the initial loading is low enough, the times to sterilize the heterogeneous population we considered are less than would be computed using a 30-minute D-value for the entire loading.

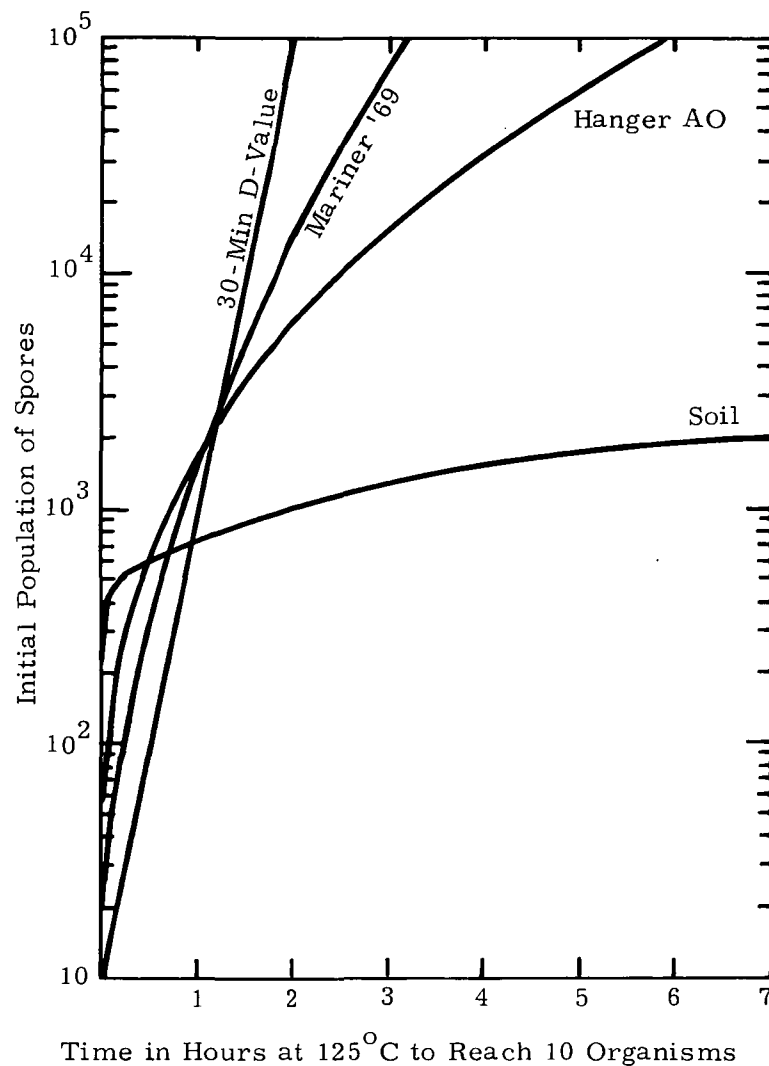


Figure II. 1.

A computer code, DEAD, which is now operable at Cape Kennedy will compute dry-heat sterilization cycles for surface organisms possessing the heterogeneity characteristics of any of the above three populations - or any arbitrarily specified heterogeneous behavior.

Subtopics and References

Separation of Organisms from Dust Particles; 21:34

Dry Heat Resistance of Sparsely Deposited Naturally Occurring Organisms;
21:19, 22:29, 23:41, 51

Model of Heterogeneous Population Survival; 21:9, 22:13, 23:9

Problems Occasioned by Heterogeneous Populations; 22:9

Natural Population Survival Data; 21:19, 23:37

Survival of PHS Resistant Strain; 20:9

Section III

Radiation Studies

The effects of ultraviolet and ionizing radiations on various biological systems have been studied. Much of this work was done in support of investigations of the effect of combined heat and radiation described in the next section.

III.A

Ultraviolet Radiation Effects

We undertook studies on the effects of ultraviolet radiation on bacterial spore survival in order to evaluate the utility of ultraviolet radiation as a potential space-craft decontamination procedure prior to vehicle launch and to determine if the ultraviolet radiation fluxes encountered in space could serve as a sterilization treatment for the launched space vehicle.

Exposure of bacterial spores to ultraviolet radiation results in a characteristic tailing of the survival curve. This tailing occurs after approximately a two-log decrease in the spore survival level. The tailing phenomenon was not an artifact due to spore clumping, i. e., protection of spores from the lethal effects of radiation by intervening spore masses, nor was tailing due to the presence of an ultraviolet radiation resistant spore sub-population. Analysis of further experiments was consistent with an interpretation of tailing as being due to the competing process of spore damage and damage repair by some repair mechanism(s) present in the microorganism.

The tailing phenomenon was also observed with spores exposed to ultraviolet radiation under vacuum and flux conditions existing in space. Continued irradiation under these conditions finally resulted in practically complete inactivation after 24-48 hours exposure. Spores found in soil from Cape Kennedy also exhibited tailing after a log decrease in survival level. However, no further reduction in survivor level was observed even after continuous exposure for six days in the simulated space environment. Soil particles were present during the irradiation treatment of the Cape Kennedy spores.

These experiments suggest that ultraviolet radiation can serve a decontamination role, but that the presence of repair processes and the possible protection of spores from ultraviolet radiation by various agents, e. g., soil particles, metal flakes, etc., mitigate against ultraviolet radiation as a spore sterilizing procedure.

Subtopics and References

Studies on Spore Inactivation; 15:16

Bacterial Spore Inactivation Studies; 16:38

Ultraviolet Radiation Inactivation of Microorganisms; 23:45

III. B

Ionizing Radiation - Analysis of Biological Radiosensitivity

Extensive effort has been directed towards identifying a specific component of a biological system which is primarily responsible for the inactivation of the system as a result of exposure to ionizing radiation. Such efforts have pointed to the nucleic acid component of a biosystem as being the prime "target" causing biological inactivation on irradiation. Recently, a correlation was made between the intrinsic radiosensitivity of diverse viruses with a parameter (C) associated with an inactivation rate term k_R . Here, the expected number of survivors, $E(n(t))$, at time t in a homogeneous population is assumed to be given by the expression

$$E(n(t)) = n(o) e^{-k_R \cdot t} \quad (III.1)$$

The k_R term appears to reflect the intrinsic inactivation response of any given biological system towards ionizing radiation. Some additional discussion of k_R can be found in Section IV-C. The term C is related to k_R by

$$k_R = C r_d \quad (III.2)$$

where r_d is the radiation dose rate in krad/hr, and C is a constant of proportionality for any given system and is equivalent to $1/D_{37}$. D_{37} is the radiation dose at which the surviving fraction is $e^{-1} = 0.37$. Table III.1 presents a total of 39 different biological entities (some 58 data points) which represent the sum of the biological systems for which both nucleic acid content and radiosensitivity data could be obtained from a search of the literature. This table indicates that the magnitude of the factor C contained in k_R is directly related to the molecular weight of the nucleic acid present in various biological systems.

A functional expression which relates C (Equation III.2) to the nucleic acid content of biosystems ranging in size from that of phage R17 ($\sim 10^6$ Daltons) to HeLa cells ($\sim 10^{13}$ Daltons) is

$$C = 2.394 \times 10^{-6} \omega^{0.480} \quad (III.3)$$

where ω is the nucleic acid molecular weight in Daltons. The constants in this expression for C were obtained by fitting the expressed form to the data in Table III. 1 using a least squares norm. The coefficient of correlation of this fit is 0.941. This degree of correlation suggests that the approximate radiation sensitivities of practically any size biological entity may be predicted on the basis of Equations III. 2 and III. 3 from their nucleic acid molecular weight. Conversely, for any given experimental value of C the approximate molecular weight of the nucleic acid present in the system under investigation could presumably be determined. Figure III. 1 illustrates the relationship between the experimentally determined values of C and molecular weights of the corresponding nucleic acids for the aforementioned 58 biological entities. The scatter in these points about the fitted line of Equation III. 3 can be attributed to a variation in the experimental conditions and procedures used in determining both the values of C and the nucleic acid molecular weights. Specifically, the variation in the energy of the radiation used in these experiments, the accuracy of dosimetry in defining the D_{37} dose, variations in test temperatures, and the difficulties in establishing a definitive molecular weight for the easily sheared nucleic acid polymers could be expected to contribute to a randomization of data about this predicted norm. An indication of the experimental variability which can be expected may be obtained by comparing the data obtained by different laboratories for ϕ X174 virus (Figure III. 1 data - 5, 6, 7, 8), Tobacco mosaic virus (Figure III. 1 data - 10, 11, 12), Newcastle disease virus (Figure III. 1 data - 18, 19), T1 phage (Figure III. 1 data - 21, 22), T2 phage (Figure III. 1 data - 29, 30, 31), and T4 phage (Figure III. 1 data - 33, 34). Notice that data for each of these virus systems are scattered about the fitted line.

TABLE III. I
Nucleic Acid Content and Radiosensitivity

Entity	Code No.	Nucleic Acid Content (Daltons)	C (krads ⁻¹)
Phage R17	1	9×10^5	1.28×10^{-3}
Tobacco ringspot virus	2	1.5×10^6	2.34×10^{-3}
Tobacco necrosis	3	1.5×10^6	1.61×10^{-3}
Tomato bushy stunt virus	4	1.6×10^6	2.34×10^{-3}
Phage ϕ X174	5	1.7×10^6	2.56×10^{-3}
Phage ϕ X174	6	1.7×10^6	3.03×10^{-3}
Phage ϕ X174	7	1.7×10^6	4.3×10^{-3}
Phage ϕ X174	8	1.7×10^6	1.25×10^{-3}
S13	9	1.7×10^6	2.56×10^{-3}
Tobacco mosaic virus	10	$1.95-2.16 \times 10^6$	5.95×10^{-3}
Tobacco mosaic virus	11	$1.95-2.16 \times 10^6$	3.33×10^{-3}
Tobacco mosaic virus	12	$1.95-2.16 \times 10^6$	3.45×10^{-3}
Polyoma	13	3×10^6	2.0×10^{-3}
Fowl plague	14	$2.2-6.0 \times 10^6$	1.08×10^{-2}
Shope papilloma virus	15	5×10^6	2.45×10^{-3}
Rous sarcoma virus	16	$0.96-1.2 \times 10^7$	5.95×10^{-3}
Phage BM	17	2.49×10^7	5.59×10^{-3}
Newcastle disease virus	18	3.2×10^7	6.18×10^{-3}
Newcastle disease virus	19	3.2×10^7	2.34×10^{-2}
Phage alpha	20	3.1×10^7	4.9×10^{-2}
Phage T1	21	3.9×10^7	1.23×10^{-2}
Phage T1	22	4.2×10^7	4.1×10^{-3}
Phage T3 (T7)	23	4.2×10^7	1.26×10^{-2}
Phage T7	24	4.2×10^7	6.66×10^{-3}
Phage 22	25	4×10^7	7.2×10^{-3}
Phage λ	26	7×10^7	9.8×10^{-3}
Adenovirus, type V	27	6.6×10^7	1.3×10^{-2}
Phage P8	28	1.0×10^8	1.3×10^{-2}
Phage T2	29	1.29×10^8	1.0×10^{-2}

TABLE III. I (CONT)

Entity	Code No.	Nucleic Acid Content (Daltons)	C (krads ⁻¹)
Phage T2	30	1.29×10^8	1.82×10^{-2}
Phage T2	31	1.29×10^8	2.1×10^{-2}
Phage T5	32	1.3×10^8	3.07×10^{-2}
Phage T4	33	1.51×10^8	1.0×10^{-2}
Phage T4	34	1.51×10^8	2.27×10^{-2}
Vaccinia virus	35	1.56×10^8	1.35×10^{-2}
Diplococcus pneumoniae	36	1.2×10^9	4.3×10^{-2}
B. subtilis var. niger Spores	37	3.15×10^9	2.34×10^{-2}
S. typhimurium	38	6.61×10^9	4.5×10^{-2}
E. coli Strain B/R	39	7.8×10^9	0.239
E. coli Strain H	40	7.8×10^9	0.326
E. coli Strain B	41	7.8×10^9	0.538
B. aertrycke	42	7.2×10^9	0.268
Micrococcus pyogenes var. aureus	43	8.5×10^9	0.268
Haemophilus influenzae	44	1.2×10^{10}	0.298
S. cerevisiae (Haploid)	45	1.49×10^{10}	0.2335
S. cerevisiae (Haploid)	46	1.6×10^{10}	0.357
Aerobacter aerogenes	47	1.7×10^{10}	0.77
E. coli Strain P6	48	2.7×10^{10}	0.127
S. cerevisiae (Diploid)	49	3.3×10^{10}	5×10^{-2}
(Triploid)	50	4.5×10^{10}	7.13×10^{-2}
(Tetraploid)	51	6.8×10^{10}	8.25×10^{-2}
(Pentaploid)	52	8.0×10^{10}	0.14 x
(Hexaploid)	53	9.6×10^{10}	0.145
Chicken Embryo Cells	54	1.5×10^{12}	3.07
Mouse (Bone Marrow in Vivo)	55	3.9×10^{12}	9.8
Guinea Pig	56	5×10^{12}	1.075
Human (Fibroblast)	57	6.3×10^{12}	14.3
HeLa	58	9.6×10^{12}	6.62

The direct relationship between radiosensitivity, C , and nucleic acid molecular weight provides further evidence for the designation of nucleic acid as the critical substrate or target being affected during radiation induced inactivation (k_R), especially since the relationship is evidenced over such a large and diverse range of biological entities irradiated under different environmental conditions (wet versus dry, vacuum versus oxygen). Figure III. 1 also reveals a unique grouping of biological systems which results from the constraints of radiosensitivity and nucleic acid molecular weight. The ordering of the groups relative to both nucleic acid content and increasing radiosensitivity is: RNA and single strand DNA viruses (●), animal and bacterial viruses (■), bacteria and yeast (▲), and vertebrate cells (◆). Similar correlations between biological complexity and radiosensitivity have been reported, but these reports have emphasized the individual and distinct character of these biological groupings. Figure III. 1 suggests that rather than being distinct and apart, these groups form a continuum of biological systems whose nucleic acid content defines not only their biological niche but also their relative radiosensitivity.

Subtopics and References

Mechanism Studies in Thermoradiation Inactivation; 20:33

Biological Inactivation by Thermoradiation; 103

Synergistic Inactivation of Viruses by Heat and Ionizing Radiation; 104

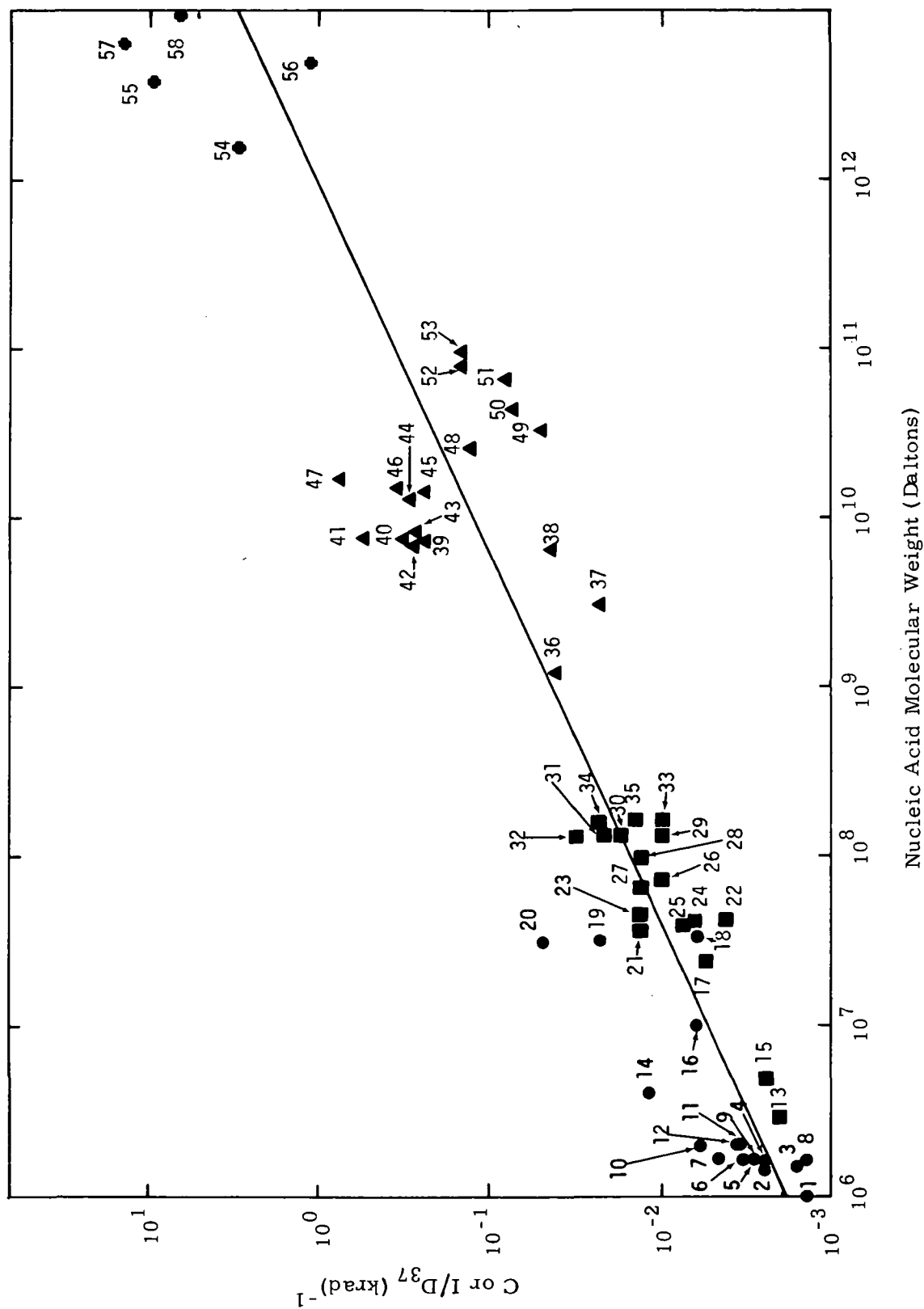


Figure III. 1

Section IV

Thermoradiation Studies

In keeping with conclusions drawn from earlier systems studies, and based partially upon speculation described in Section II, this activity represented an attempt to develop a reliable sterilization method which offered a hope of being less severe to hardware than dry heat. This activity was begun as concern was being expressed over the potential severity of dry heat sterilization.

IV.A

Synergism and Other Properties of Thermoradiation

This study was initiated to find alternatives to dry heat sterilization of planetary spacecraft. Extensive literature searches in the area of radiation sterilization resulted in a considerable amount of conflicting data, but at least an indication of promise under certain conditions of irradiation. A systematic investigation of bacterial inactivation was made with particular attention to the control of all experimental parameters. We located small ovens suitable for biological use, modified them for bacterial samples and developed remote temperature control systems to avoid long term radiation deterioration of controls. Additional equipment was designed to maintain precise moisture conditions in the ovens during radiation exposure. A technique using foil disks as a substrate for biological samples was developed to facilitate radiation exposure and transport of samples between laboratory clean rooms and radiation facilities. Several experiments were conducted to determine that these foils do not differ significantly from other substrates.

Base line experiments were performed using Bacillus subtilis var. niger spores. Inactivation rates of this organism were determined during dry heat treatment and gamma irradiation. These results were then compared to inactivation using simultaneous combinations of heat and γ -radiation (thermoradiation). In this way, a rather systematic assessment could be made of the synergism alluded to in some journal articles. This assessment was made over a temperature range from 60° to 125°C. During this investigation, it was discovered that for each specific temperature, there was an optimum gamma dose rate which maximized synergism. Generally the dose rates used by other investigators were found to be far in excess of this optimum, resulting in marginal benefits from a combined heat/radiation treatment. At room temperature, some lower dose rates can accomplish the same degree of sterilization with about one-half the dose required at some higher dose rates. Even greater benefits are available at temperatures above room temperatures. Careful selection of dose rates can then afford substantial reductions in the radiation required for sterilization.

In addition to the dose rate effect determination, we evaluated many other parameters that might be significant in spacecraft sterilization. The population density was

found to have a minor effect on inactivation. High populations reduced the inactivation rate, thus requiring a higher dose/time-at-temperature for a given population reduction. A population increase by 10^3 required 50% greater radiation exposure levels. Relative humidity of the surrounding air was found to affect dry heat inactivation significantly, whereas, over large ranges of RH, almost no effect was observed in thermoradiation inactivation. In order to evaluate the inactivation of contaminants buried in materials, an experimental series was performed with bacterial spores encapsulated in methyl methacrylate plastic. In this case, dry heat inactivation at 105°C required 30 hours per log population reduction; thermoradiation at 105°C and 12 krad/hr required only 5 hours per log. Plans for sterilization of spacecraft in a dry nitrogen atmosphere necessitated the evaluation of dry heat, radiation, and thermoradiation inactivation in N_2 instead of air. Dry heat inactivation was more efficient in N_2 than in air. Radiation was less efficient in N_2 as was thermoradiation in N_2 at the higher dose rates. At optimum dose rates described earlier, however, there was little difference between thermoradiation in air or in N_2 .

Gamma radiation, using 60-cobalt was used for the preceeding experimentation because of its availability, deep penetration and relatively low cost. We did, however, perform experiments using high energy x-rays to permit comparisons to be made with base line gamma inactivation. We used the Hermes II, Sandia high energy pulse x-ray machine in conjunction with the ovens and other equipment used in the gamma experiments. X-ray experimental results, where dose and time at temperature were matched to the base line gamma work, showed no difference in the inactivation rates by x-rays as compared to gamma rays. (These experiments were performed both at room temperature and 105°C).

After assessment of the parameters which may alter the inactivation rate of Bacillus subtilis var. niger, the next step was to determine the applicability of the process to other bacterial contaminants. The toughest organisms we could find were certain naturally occurring (not subcultured) spores in soil. These were 100 times as heat resistant as B. subtilis spores and 3 times as resistant to radiation. At 125°C , 30 hours of dry heat was required to reduce the population by one log. When 50 krads of radiation were combined with the dry heat, only one hour at 125°C was required. Other organisms investigated were Bacillus pumilus and Micrococcus radiodurans. Both were inactivated synergistically.

Besides the above microbiological studies, additional activities included: a feasibility study of radiation facilities for spacecraft sterilization (with Reactor Source Applications Division 5221); the evaluation of radiation effects on electronic components (with Radiation Effects Organization 1930); the evaluation of radiation effects and mechanical and physical properties of polymers (with Solid Dynamics Research Organization 5160 and Organic Materials Research Organization 5510); a study of alterations and differences in individual spore morphology using electron microscopy (with Organization 1910); and a study to determine the potential for enhancing physical and/or mechanical properties of plastics by thermoradiation (with Organization 5510). Generally, there were no adverse effects to electronics at the dose levels proposed for spacecraft sterilization. Material degradation at these radiation levels was also found to be minimal. The study of mechanical/physical property enhancement, although promising, has not progressed sufficiently to draw specific conclusions.

Subtopics and References

Gamma Radiation Inactivation of Bacillus subtilis var. niger Spores; 13:27, 18:11, 20:24, 23:35, 44, 46, 70, 71, 72, 86, 103

Dry Heat Inactivation of Bacillus subtilis var. niger Spores; 13:27, 18:12, 31, 44, 46, 70, 71, 72, 86, 103

Thermoradiation Inactivation of Bacillus subtilis var. niger Spores; 15:12, 16:33, 17:10, 18:12, 19:21, 20:21, 22:21, 44, 46, 70, 71, 72, 86, 87, 89, 103

Dose Rate Effect on Radiation and Thermoradiation Inactivation; 15:12, 17:11, 18:12, 19:21, 20:19, 20:23, 22:21, 23:35, 44, 46, 70, 71, 72, 86, 87, 89

Clumping Effect of Bacillus subtilis var. niger in Thermoradiation; 14:37, 15:8, 17:18

Effects of Relative Humidity on Dry Heat and Thermoradiation; 14:38, 16:28, 20:9, 48, 49, 72

Effect of N₂ Atmosphere on Radiation, Dry Heat and Thermoradiation Inactivation; 19:9, 20:22, 23:29, 86, 87, 89

X-Ray Inactivation of Bacillus subtilis var. niger Spores; 20:12

Dry Heat and Thermoradiation Inactivation of Bacillus pumilus Spores; 13:30

Dry Heat, Radiation and Thermoradiation Inactivation of Naturally Occurring
Spores In Soil; 20:10, 22:22, 23:34

Radiation Effects on Materials and Components; 17:19

Data Analysis; 21:53, 79

Facilities for Thermoradiation Sterilization of Spacecraft; 17:19

IV. B

The Mechanistic Basis of Synergism

Studies were undertaken to define the mechanistic basis of the synergistic inactivation of spores in a combined heat and radiation environment (thermoradiation). The approach used in this study was to consider a spore as a complex biological system composed of various classes of biopolymers, i. e. , nucleic acids, proteins, lipids. The method selected for studying the mechanism of the synergistic inactivation of spores by thermoradiation was to study the individual response of each class of biopolymer to thermoradiation, and to observe if any correlations existed between the inactivation of a given class of biopolymer and the inactivation of spores in a thermoradiation environment. An important result of this research effort was the realization that apparently all biological systems respond to thermoradiation in a synergistic manner. This conclusion was substantiated in the laboratory by our studies on lysozyme, T4 bacteriophage, bacterial spores, and bacterial vegetative cells. All have shown a synergistic response to thermoradiation and all have shown a temperature dependent radiosensitivity. A review of the available literature shows that other biological systems exhibit this same synergistic response to thermoradiation. The uniform response of biological systems - from enzymes to human cells - to thermoradiation suggests that a basic principle in radiobiology is being observed.

Because of this potential fundamental character of thermoradiation synergism, research efforts concerned with understanding thermoradiation phenomena through analysis of thermoradiation effects on biological systems were undertaken. Specifically, a study of the inactivation of T4 bacteriophage, a double-stranded DNA virus, by thermoradiation yielded a three-term model which successfully described the inactivation process. This model described the rate of inactivation as

$$k = k_T + k_R + k_{TR} \quad (IV.1)$$

This expression assumes a "logarithmic" type of survival curve which has been observed experimentally for the majority of dose rates used.

In this analysis, k_T is the thermal inactivation rate parameter, approached rationally, as described in Section II, k_R is the temperature independent radiation inactivation rate parameter associated with bond breakage or other point defects in the critical substrate resulting from the action of ionizing radiation described in Section III, and k_{TR} is a temperature dependent inactivation rate for a chemical process sponsored by the action of the radiation. If a biological entity is placed in a composite heat and radiation inactivating environment (thermoradiation), the three parameters, k_T , k_R , and k_{TR} will all contribute to the overall inactivation rate k (Equation IV. 1). Similarly, in a heat or in a low temperature radiation environment alone the only parameters contributing to k would be k_T or k_R respectively.

The utility of the three-term model in analyzing the thermoradiation inactivation phenomenon should be stressed. For example, three characteristic properties of thermoradiation are described by the model:

1. The inactivation of biological systems by thermoradiation is synergistic in nature. The term k_{TR} of Equation IV. 1 describes the absolute amount of synergism, since the sum of the k_T and k_R parameters is less than the overall inactivation rate parameter k ; and therefore, an additional inactivation parameter, k_{TR} , was defined which serves to represent the thermoradiation component providing for this synergistic inactivation. A rational basis for the k_{TR} term has been presented.
2. A second feature of thermoradiation inactivation is that the radio-sensitivity of the biological system varies with temperature. As the temperature during irradiation is increased, the rate of inactivation also increases. This increase in the composite thermoradiation parameter k is only partially due to the contribution from the thermal inactivation term k_T . The balance of the increase in k is defined by the increase in the parameter k_{TR} as the temperature is increased. The thermal inactivation properties of the biological system under study will determine the magnitude of the contribution of k_T to the increase in k with temperature. The k_R parameter is a temperature independent parameter and will make no contribution to the increasing value of k with temperature.

3. The detailed studies on the inactivation of B. subtilis spores by thermoradiation described in the previous subsection have indicated a third characteristic of thermoradiation. The degree of synergism, and hence the magnitude of the k_{TR} rate parameter, is apparently a nonlinear function of the radiation dose rate. More limited studies on the effect of dose rate on T4 bacteriophage inactivation also exhibit this phenomenon. The detailed expression for k_{TR} , given below, shows this dependence. Verification of the general nature of this response to thermoradiation must await more complete study of dose rate effects on the thermoradiation inactivation of general biological systems.

Therefore, it appears that the effect of thermoradiation on biological systems can be characterized by synergism, by a temperature dependent radiosensitivity, and tentatively, by a nonlinear dependency of the k_{TR} parameter on radiation dose rate. Each of these attributes is germane to defining the extent of biological inactivation caused by exposure to thermoradiation, and each has been successfully incorporated into the analytical three-term model which describes the thermoradiation inactivation process.

In Equation IV.1, the third inactivation rate parameter, k_{TR} , is believed to represent the reaction or intervention of free radical species in biological inactivation. The dose rate dependence may be thought of as stemming from the dependence of the concentration of free radicals on the dose rate, and the temperature dependence is present due to the effect of temperature on the rate at which the dominant radicals react with the critical substrate and/or the changing configuration of this substrate. An expression which has been found to accurately represent k_{TR} for dry spores, vegetative cells and bacteriophage is

$$k_{TR} = r_d^{\beta/T} e^{\alpha} e^{-\gamma/RT} \quad (IV.2)$$

or

$$k_{TR} = e^{\alpha} e^{-(\gamma - \beta R \cdot \text{Log } r_d)/RT} = e^{\alpha} e^{-\Delta H/RT} \quad (IV.3)$$

where α , β , and γ are constants and ΔH is defined by

$$\Delta H = \gamma - \beta R \cdot \text{Log } r_d \quad (IV.4)$$

A listing of the parameters which define the temperature dependent radiosensitivity term, k_{TR} , for each biological system for which thermoradiation data could be obtained is presented in Table IV. 1. Figure IV. 1 shows plots of k_{TR} as a function of temperature, presented for the various types of systems of Table IV. 1, ranging from ϕ X174 virus to HeLa cells. Notice that there is a great difference between the temperature dependences of the radiosensitivities of the wet and dry systems. Figure IV. 1 also shows that the placement of the k_{TR} curves relative to a temperature range is generally dependent on the nucleic acid content of the system, i. e., the HeLa cells have a non-zero k_{TR} at a much lower temperature than do the viruses.

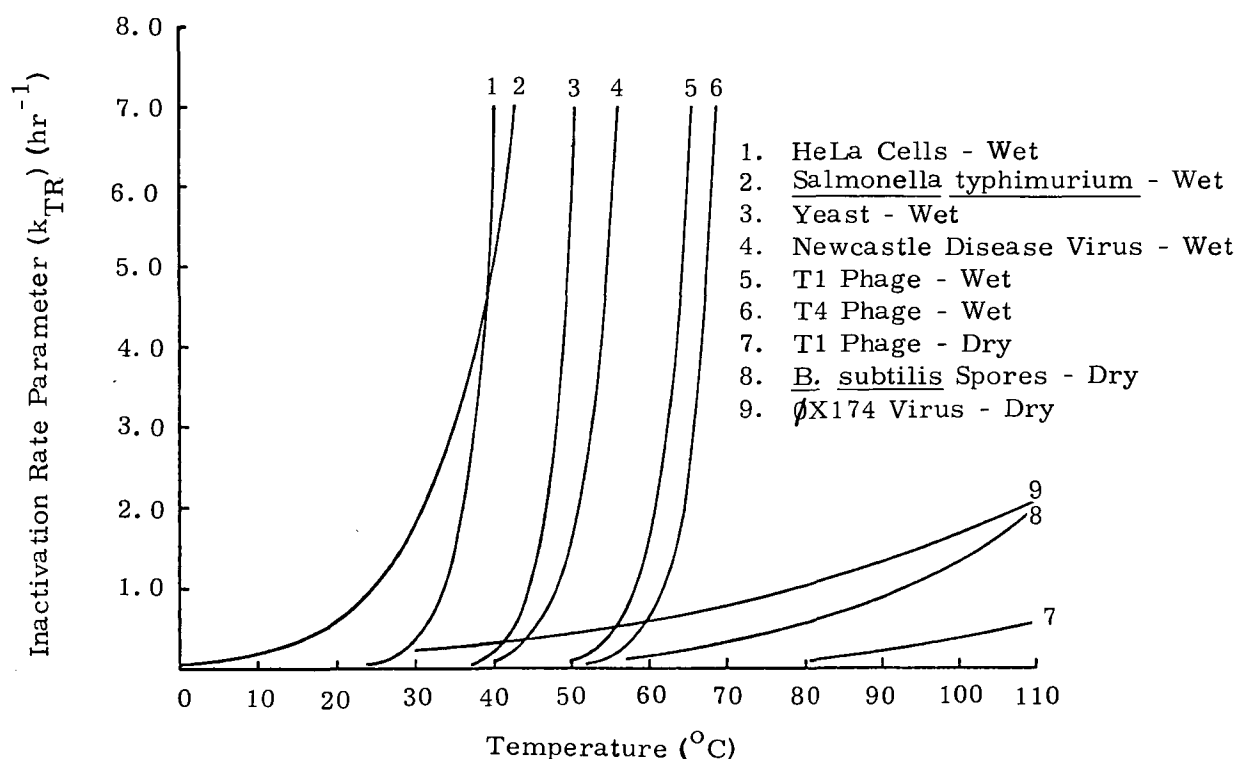


Figure IV. 1. A comparison of the inactivation rate parameters (k_{TR}) for a wide range of biological systems. The radiation dose rate for each system is presented in Table IV. 1.

TABLE IV. 1

Factors and Parameters Defining Thermoradiation Inactivation

Entity	$\phi X174$	Newcastle Disease Virus	T1		T4	<i>B. subtilis</i> var. <i>niger</i> Spores	<i>Salmonella typhimurium</i>	<i>S. cerevisiae</i> (Haploid)	HeLa
Radiation Conditions (krad/hr)	1500, Co ⁶⁰	291, Co ⁶⁰	25, X-ray		30.6, Co ⁶⁰	30.6, Co ⁶⁰	300, Co ⁶⁰	24.8, X-ray	1.512, X-ray
Physical Conditions	Dry, Vacuum	Wet	Dry	Wet	Wet	Dry	Whole Egg	Wet	Wet
K_T									
$\Delta S^\ddagger$ (cal/deg · mole)	-6.33	22.4	0	208	245.45	12.63	-	-	-
$\Delta H^\ddagger$ (cal/mole)	26763	30900	28000	95000	107416	33590	-	-	-
k_{TR}									
α	9.13	83.15	22.50	91.4	95.10	16.27	34.62	109.94	90.50
$\Delta H^\ddagger$	6400.7	53114.3	17463.3	60238.2	63289.9	11863.08	20509.8	69457.8	55190.1
k_R									
C	0.00125	0.00618	0.0043	0.0039	0.0227	0.0234	0.045	0.2335	6.62
Nucleic Acid Vol. Weight (Daltons)	1.7×10^6	3.2×10^7	4.2×10^7		1.5×10^8	3.75×10^9	6.61×10^9	1.51×10^{10}	9.6×10^{12}

In summary, these efforts have yielded a more complete understanding of thermoradiation through the use of a three-term model. The parameters which constitute this model have been individually studied and certain characteristics of these parameters have been identified. The interrelations between the parameters have also been subjected to inquiry. Such interactions have been found to be of major importance in both the experimental observation of a synergistic effect and the optimization of a synergistic response for given conditions of temperature or radiation dose rate.

Efforts have also been made to develop a model of thermoradiation inactivation which, as nearly as possible, may be derived from first principles.

Recent work by Freifelder, and Freifelder and Trumbo, is very suggestive in this respect. Freifelder has observed that apparently two modes of inducing double-strand breaks (dsb's) in double-stranded DNA exist:

- One a "direct" effect which is temperature independent.
- One apparently caused by single-strand breaks (ssb's) which ultimately combine to cause a dsb in a temperature dependent fashion.

This latter effect is part of a theory of irradiation damage to double-stranded DNA which ascribes part of the damage to single-strand breaks (ssb's) occurring in opposite strands of the DNA backbone within a distance h (nucleotide units) of one another - causing a double-strand break. Some investigators have suggested that the ssb's in this h-theory are caused by free radicals, and there exists experimental evidence to support such a hypothesis.

If it is assumed that a dsb is equivalent to "sterilization", that ssb's are free radical mediated, that the above "theory" obtains, and that the ssb $\rightarrow$ dsb mechanism is that associated with synergism, then an expression for k_{TR} can be derived from molecular considerations. This expression:

- i) Is consistent with the in vitro DNA breakage data of Freifelder
- ii) Is consistent with the known form of behavior of k_{TR} described above.

One possible means to experimentally check the assumption that k_{TR} is the survival counterpart of "h-theory", rests with Freifelder's observation that ssb $\rightarrow$ dsb mechanisms are severely curtailed in the presence of L-histidine. Thus, if organisms exhibit less or no synergism in a thermoradiation environment in the presence of L-histidine, this would constitute some experimental evidence that k_{TR} represents molecular behavior compatible with h-theory. Such data have been recently obtained.

Subtopics and References

Mechanism Studies; 19:23, 20:33

Modeling; 15:22, 16:8, 17:20, 18:28, 20:25, 21:25, 23:17, 44, 46, 103

Viruses; 103, 104

Bacteriophage; 102, 103

Molecular Speculation; 23:17

IV.C

Consultation

In the area of thermoradiation, we have provided information, consultation, assistance in experimentation or data to many persons and organizations. Some of these activities are described below.

A large effort was made at the request of the Planetary Quarantine Officer to familiarize project personnel on the potential and probable effects of thermoradiation. Presentations were made on the use of thermoradiation for sterilization of spacecraft, how sterilization cycles could be optimized and the probable radiation doses required. In addition, the probable radiation effects on spacecraft as a result of these sterilization cycles were assessed and found to be negligible for spacecraft that are designed for such cycles. Finally, a feasibility study of radiation facilities for spacecraft sterilization determined that the additional cost associated with radiation would be quite reasonable for properly designed facilities. Representatives from NASA Headquarters, NASA Centers, and major contractors for the Viking Program were briefed.

The U. S. Army Medical Research and Development Command requested information on spacecraft sterilization technology with possible application to field sterilization of medical equipment. Possible use of thermoradiation in this area was studied and such information provided.

An entire session on thermoradiation sterilization and its implications was presented at the 1971 annual meeting of the American Society for Microbiology. This presentation involved persons from Sandia, private industry, the Public Health Service and the Food and Drug Administration.

We collaborated with the Public Health Service, Phoenix Laboratories, in dry heat and thermoradiation experimentation using naturally occurring organisms in soil. Thermoradiation was found highly effective against dry heat resistant soil spores. In addition, Sandia electron microscopes were used in identification studies of these naturally occurring soil organisms and also in studies of the morphology of Pseudomonas aeruginosa occurring in distilled water intravenous kits. The cell morphology of the

heat resistant soil spores compared favorably with electron micrographs of specific spore species appearing in the literature, but PHS personnel found biochemical differences.

At their request, we consulted with the Metropolitan Sanitary District, Chicago, Illinois, on the effects of thermoradiation on bacteria and how it might resolve some of their problems in waste water treatment. Not only might thermoradiation offer a way to sterilize huge volumes of sewage with reasonable efficiency, but it would appear, as a side benefit, that radiation favorably influences the settling rate of many solids.

Section V

Chemical Studies

A number of studies that might be classed as being of a chemical nature were undertaken with one of two basic objectives in mind: the development of more effective or useful chemical sterilants, or the development of more basic understanding of physical sterilization methods. Some of these studies are described in this section.

V.A

Biological State and Chemical Sensitivity

Insight into the mechanism of spore inactivation by various treatments may be obtained by studying the effect of such treatments on the stages involved in the transformation of a spore into a vegetative cell. These states, together with some of their properties, are as follows:

<u>Dormant</u>	<u>Germinated Spore</u>	<u>Vegetative Cell</u>
1. Heat resistant	1. Loss of heat resistance	1. Viable bacterium
2. Refractile	2. Decrease in optical density and refractility	2. Cell reproduction
3. Dormant metabolism	3. Macromolecular synthesis	

Inactivation treatments can function either by interfering with the spore's ability to transform into the germinated state or by interfering with the germinated spore's capacity for macromolecular synthesis and cell division. Since these stages are biochemically and physiologically distinct, information about the mechanism of inactivation by various treatments must begin by identifying the spore state at which the treatments exert their influence.

An inactivation criterion which defines spore inactivation in terms of a lack of colony-forming ability is somewhat specious, since a given treatment could conceivably interfere with only the spore's capacity to be transformed into the germinated spore state and not affect the potential of the spore to yield a vegetative cell with a viable reproductive capacity. Therefore, studies on the mechanism of spore inactivation by various treatments began by identifying the spore state at which the inactivating treatments exert their influence.

Initially, it was found that heat and/or radiation did not seem to interfere with the mechanisms of spore germination. This conclusion was based on the fact that, after treatment, spores retained their characteristic optical density change in a germination medium, although the time period required for germination may at times be lengthened.

Additionally, it was observed that chemical agents were capable of affecting the spore germination process. Low levels of aliphatic or aromatic alcohols were found to inhibit the process of spore germination, the extent of germination being directly related to the amount of alcohol present in the germinating media. The inhibition of the spore germination was found to be completely reversible since removal of the alcohol from the spore environment allowed germination to proceed.

Formaldehyde in aqueous solution was another chemical agent capable of influencing the transition from spore into vegetative cell. Aqueous formaldehyde was found to exert both a sporostatic and sporocidal effect on B. subtilis spores. The sporostatic effect was due to the reversible inhibition of spore germination occasioned by aqueous formaldehyde. This result was analogous to that obtained with aliphatic and aromatic alcohols. Aqueous formaldehyde differed from the alcohols in that it also was sporicidal. This sporicidal property of aqueous formaldehyde was found to be a highly temperature dependent process. Aqueous formaldehyde was therefore capable of interfering with both the germination and outgrowth mechanisms involved in the conversion of a spore into a vegetative cell. In the course of these investigations, solid and liquid forms of formaldehyde were developed which possess the disinfectant properties of formaldehyde without the irritating and noxious properties normally associated with formaldehyde disinfection. Both of these new forms of formaldehyde are currently in the process of being patented by NASA.

Subtopics and References

Germination Inhibition; 15:16, 16:41, 101, 105

Alcohol; 17:27, 21:45, 101

Formaldehyde; 21:45, 105

V.B

DNA Involvement in Sterilization

8

Some of the work reported in Sections II, III, and IV yielded indirect evidence for the hypothesis that DNA is the critical substrate involved in heat and/or radiation sterilization. For example, the dependence of k_R (Section III) on dominant nucleic acid molecular weight constitutes such evidence, as does the observation that the temperature at which k_{TR} (Section IV) becomes significant appears to depend upon the molecular weight of the DNA of the cells being treated. Also, in developing a model for the dry heat sterilization of heterogeneous populations (Section II) and a "first principle" model of k_{TR} (Section IV), DNA was assumed to be the critical substrate. Particularly to gain more confidence in these latter two activities, more nearly direct evidence of DNA involvement in the sterilization processes was desired.

One of our first attempts at assessing the role of DNA in sterilization was the examination of "long term" survivors of B. subtilis var. niger in lethal environments. We took samples just preceeding samples in which there were no survivors from thermoradiation treatments, and examined survivors from the former samples for mutational state. Only a limited number of such samples were taken over a six-month period, but in each case the survivors were found to be genetically defective. The genetic defects were not the same in all cases, but one of the most notably consistent ones was evidenced by the requirement for glutamic acid in the recovery medium in order that strong growth take place. The original stock had no such requirement prior to a thermoradiation treatment. Thus it could be concluded that in all cases examined, damage to the DNA of B. subtilis var. niger spores was done by thermoradiation treatments.

At the same time as the above experiments, means for obtaining more direct evidence of DNA involvement in sterilization were being sought. It was known that at least some of the genetic damage caused in long term survivors of thermoradiation could be corrected through the process of transformation. In this process, for example, mutants requiring the addition of a specific amino acid to their growth medium before growth will take place, when this is not true of the associated wild type, can be "transformed" again to wild type (vis-a-vis this property) by exposure to DNA

extracted from wild type organisms under certain conditions. Because of this ability to transform "marginal survivors," into more "normal" organisms, it was speculated that possibly lethal damage could be corrected through a similar transformation process. If this could be done, it would certainly offer the direct evidence of DNA involvement in sterilization that was being sought.

Work reported in the previous subsection indicating that spore germination takes place after heating, even though subsequent growth does not, suggested that one might try transforming experiments on spores after dry heat sterilization treatments since their coats become vegetative-like in a germination medium after such a treatment, hopefully permitting DNA to pass through them. Such experiments have been begun. Problems involved in DNA extraction, quantification, and purification have been overcome. Preliminary results give an indication that recovery is greater in such transformed organisms than in controls, but the transformation efficiency is quite low and appears to be related to DNA concentration. Methods are being studied to increase this efficiency, but they will necessarily differ from those typically used in transforming amino acid deficient mutants, since the latter methods require a culture in the log phase of growth.

Section VI

Fine Particle Physics

This activity represents both a continuation of research by some members of this group prior to work on planetary quarantine and also a field of considerable importance in measuring, estimating and predicting the bioburden on spacecraft surfaces.

VI.A

Experimental Programs and Associated Developments

The study of fine particle physics provides information on how spacecraft surfaces accumulate and retain particles to which microorganisms become attached, and, as a corollary, how particle burdens may be influenced. This information is extremely important, especially in the case of spacecraft surfaces that will not withstand the use of liquid solvents to reduce the final microbial burden before final sterilization; particularly if these surfaces may be exposed to high humidity, even for short periods of time. This is made even more critical with the finding by the Public Health Service of extremely hardy naturally occurring microorganisms.

The experimentation in fine particle physics was done in the following areas: airborne particle generation and behavior characteristics, surface particle retention mechanisms including the significant effects of relative humidity, and airborne biological contamination and its relationship to particles. The measurement of surface microbial loading is described under the Vacuum Probe in the following subsection of this report. In nearly all instances, special purpose equipment was developed or modified for use in these experiments.

One of the problems initially encountered in this area involved the elimination of clumping effects of particles deposited on surfaces, since clumping might affect the sterilization of surface organisms. An electrostatic deposition device was developed to provide a means of depositing a monolayer of particles (unclumped) and to closely control the density of particles over the surface area. Subsequent studies using this device showed only modest difference in thermal sterilization characteristics between clumped and monolayer bacterial populations.

In order to simulate spacecraft assembly environments under controlled conditions, several methods of microbiologically tagging dust particles and disseminating them into a controlled environment over long periods of time were developed using a spinning disc aerosol generator and an acoustic particle disseminator. In this way, particles of known size and with known mean numbers of bacteria attached to them could be uniformly dispersed in air for determining particulate fallout patterns and densities.

The Andersen Sampler, used for measuring airborne microbial contamination, has one discernable limitation when used in conjunction with these studies. The quantity of air needed to be drawn through the sampler in relatively clean spacecraft assembly areas in order to obtain reliable data is sufficient to dry the agar medium in the sampler, thus preventing long term tests. To overcome this deficiency, a membrane filter positioned over a flat piece of open-celled foam saturated with a saline solution was used in place of the agar. The sampler was further modified to include an automatic deionized water supply to keep the filter damp. This development therefore facilitated long term sampling of air having a very light microbial density.

It has been shown that the procedure required to sterilize by dry heat is somewhat dependent on the initial microbial loading of a surface. With this in mind, experiments were conducted to determine the effect of ambient relative humidity on surface particle retention. Dust particles were uniformly deposited on polished metal foils and were photographed before and after exposure to relative humidities ranging from 33% to 100% and after a controlled dry nitrogen blow-off operation. The results showed that particles exposed to higher humidities for even a few minutes sometimes dissolved into a multitude of smaller particles, and in all cases exhibited a much stronger bond to the surface than those particles exposed to low humidity conditions. These results emphasize the need for low relative humidity in spacecraft assembly areas if the initial bioburden is to be limited to levels commensurate with present dry heat sterilization techniques.

The information and data obtained from these studies were used as input for verification of models and for specific recommendations to NASA concerning cleanliness levels required to meet stated Planetary Quarantine objectives.

Subtopics and References

Fine Particle Behavior on Surfaces; 1:11, 2:4, 3:10, 6:14
Electrostatic Deposition Device; 8:15, 9:28, 119
Spinning Disc Aerosol Generator; 8:16, 10:11
Mixing Spores and Inert Particles; 8:17, 9:26, 10:9
Acoustic Particle Disseminator; 9:27, 10:12, 40, 64
Modified Andersen Sampler; 16:35
Fallout Surface Particle Retention; 20:55
Effects of RH on Surface Particle Retention; 21:29, 123

VI.B

The Vacuum Probe and Other Bioburden Sampling Techniques

The development of the vacuum probe was motivated by the need for a microbiological surface sampling device with the capability for sampling large irregularly shaped surface areas that are lightly loaded with microorganisms. The intended use of the instrument was to sample clean surfaces in laminar flow clean rooms, but the device has been utilized for sampling surfaces in other clean environments. The vacuum probe was developed, fabricated, and tested by us. It operated on the principle of turbulent sonic airflow at a critical orifice, making the removal of small particles easier than it is by traditional frictional methods. In all tests the vacuum probe removed a mean of never less than 89% and assayed a mean of never less than 67% of bacterial spores approximately 1 μ in length settled on smooth surfaces which were free of viscous films. Detailed machine and assembly drawings and instructions for use of the probe were published.

The vacuum probe has since been used for sampling surfaces of spacecraft, hospitals, laboratories, etc., for microbial contamination. It has also been used for monitoring particle contamination on photographic film. The vacuum probe is now being commercially produced.

To complement the capability of assaying surfaces provided by the vacuum probe, a means was developed for determining the spatial distribution of organism bearing particles on irregularly shaped experimental surfaces. This was made possible by the development of a device that could spray molten agar onto surfaces, thereby providing nutrient for organisms on those surfaces. To prevent the agar from drying before colony development could take place, Krylon, or a similar material, was sprayed over the agar.

Subtopics and References

Vacuum Probe Development; 2:3, 3:9, 41, 43, 66, 113

Vacuum Probe Testing; 5:12, 6:13

Improved Model; 8:14, 9:28

Agar Spray Technique; 57, 68

VI.C

Models

Modeling in the area of fine particle physics has largely been confined to the special case treating microorganisms in environments and on surfaces.

Most studies that have been made to determine the numbers and kinds of microorganisms which are on surfaces in various environments are based on microbiological assays of surfaces. Since it is not feasible to assay an entire spacecraft, sampling techniques must be employed. These techniques utilize direct assays of small portions of the surface of the spacecraft and a characterization of the environment around the spacecraft. Because of this, a mathematical model which yields a complete probability distribution for the bioburden is necessary in order for one to know the number of samples necessary for making bioburden estimates at a given confidence level. Such a model has become known as an estimation, or direct assay, microbial model.

In most cases actual samples cannot be taken on all surfaces right up to the actual start of sterilization. It is then necessary to extrapolate forward in time from previous estimates (based on surface assay data) using environmental data. A model which has this ability is known as a prediction model.

At a given point in time, it is reasonable that the prediction model should have the same abstract form as the estimation model - making the estimation model a special case of the prediction model from which sampling requirements at a specific time may be derived.

In the development of such a model, it was observed that there are three basic entities that determine the number of organisms on a surface at a given time. First, organisms should be viewed as occurring in clumps, termed viable particles. Roughly, the distribution of the number of organisms per clump is one of these basic entities. Then the rate of deposition of these clumps (from all sources) onto the surface and the rate of their removal (in any way) are the other two entities. Intuitively, knowledge of these three entities over a long period of time should be sufficient to allow one to estimate or predict the bioburden on a surface at any given time. The primary

purpose, then, of a bioburden model is to describe the relationships which exist between these three basic entities and the bioburden on the surface. There are secondary goals related primarily to sampling and program planning which would be achieved if a basic model were available.

In the general prediction model that was developed, the probability that n microorganisms are on a surface at any time t takes the form

$$P_n(t) = \sum_{k=0}^{\infty} \frac{(H(t))^k e^{-H(t)}}{k!} Q(n, k) \quad (VI.1)$$

where

$H(t)$ is the mean number of viable particles on the surface at time t , and $Q(n, k)$ represents the probability that k particles have n viable organisms on them. The function $Q(n, k)$ can be computed from the probability distribution of the number of microorganisms on a single particle.

The function $H(t)$ is related to the assembly parameters

$\lambda(t)$, the particle deposition rate (from all sources), at time t , and $\mu(t)$, the particle removal fraction (by all means), at time t

through the differential equation

$$H'(t) = \lambda(t) - \mu(t) H(t) \quad (VI.2)$$

Thus, in specific situations, the distribution of the number of microorganisms per particle, $\lambda(t)$, and $\mu(t)$ may be measured or estimated and $P_n(t)$ subsequently determined by solving Equation VI. 2 and substituting into Equation VI. 1. Using data obtained at Cape Kennedy, we found this approach to work quite well for predicting bioburdens at times beyond those at which surface sampling was available.

Additionally, Equation VI. 1 has been successfully used as an estimation model, when $H(t) \equiv H$ is simply a number at a specific point in time, to determine sampling protocols for the Lunar Quarantine Program described later in this document.

This model has many desirable properties. Among these are:

1. It provides a closed form description of both the estimation and prediction models.
2. There is compatibility between the estimation and prediction models.

3. It provides a method for varying the parameters in the model on the basis of surface samples.
4. A confidence interval about the predicted bioburden value can be assigned.
5. Clumping is taken into account and the distribution of the number of microorganisms per clump can be specified.
6. The model exhibits the plateau effect that is always observed.
7. Sampling protocol can be established.
8. It has been found to agree with data.
9. It is computerized and extremely easy to use.
10. It presents a framework in which contractor estimated burden hypothesis testing may be done by the Planetary Quarantine Office with minimal sampling.

In addition to this work, a simple model for deriving sampling requirements for bacterial identification purposes was developed and used in the Lunar Program. This was referred to as a qualitative sampling model.

Subtopics and References

Estimation Model; 8:8, 10:7, 14:30, 16:17, 17:47, 18:10, 73, 75, 78, 82

Prediction Model; 15:27, 16:17, 17:47, 18:60, 75, 82

Agreement With Data; 8:8, 16:17, 17:47, 18:60, 75, 82

Use in the Apollo Program; 10:5, 10:7, 73, 78

Qualitative Sampling Model; 10:5, 13:13, 74

Section VII

Lunar Systems

This section describes work done to enable NASA lunar quarantine policy to be carried out. This activity included a complete systems study of the problem area, development of models in specific subareas, and the development of an information system whose output was that information required by NASA policy.

VII.A

General Systems Study and Computerization

NASA adopted a policy concerning the documentation and/or reduction of terrestrial microbial life which might be transferred to the Moon as follows:

"Microbial life landed on the Moon by outbound lunar missions shall be identified, quantified and, insofar as possible, located in order that life on the returning spacecraft and samples of lunar materials may be more easily identified as of terrestrial origin if that is the case."

Various methods of meeting this requirement were analyzed, resulting in the decision that an information system would most efficiently satisfy the requirements of the policy. A Lunar Information System was designed by Sandia Laboratories to store, categorize, and analyze the data collected by the Public Health Service so that the information required by the above policy is continuously available to the Planetary Quarantine Officer.

The Lunar Information System design resulted from a comprehensive systems study of the lunar "quarantine" problem. The resulting system consists of four major interacting portions:

1. A preparation subsystem which specifies the way in which spacecraft data, sampling data on the spacecraft, and the spacecraft environment are stored, and file locations to these data. It also handles the specific configuration of men and equipment assigned to a specific Apollo flight as well as launch dates and expected landing coordinates. Environmental sampling sites are also specified through this subsystem.
2. A data storage subsystem which stores microbial burden data, calls appropriate data processing subroutines, and stores the calculated results in both permanent files and temporary disk files.
3. A lunar inventory subsystem which contains bioburden data on all flights which have reached the lunar surface, continually updates

the estimated burden of terrestrial organisms on the Moon, predicts the density of these organisms at any specified lunar coordinates at any time, and computes the likelihood of the contamination of lunar samples.

4. A communication subsystem which provides a language for requesting necessary information from the system, with a format and user guide for using this language.

A number of programs make up these subsystems. FILE is the program for the preparation subsystem. It establishes and maintains the files and directories used in the rest of the system. The quantitative data within the system are handled by a data storage program called DAST. Spacecraft bioburdens at launch and at the lunar surface are computed by this program. A reproduction program, REPRO, has the capability of being able to reproduce any file maintained by the Lunar Information System. QUAL is a program which accepts the results of qualitative (biochemical and morphological) tests performed for the purpose of identifying colonies taken from Apollo modules, identifies each colony using a Public Health Service identification system, and prints and stores the type of colony and its test results. The Qualitative Summary Program (QUALSUM) uses the output file from QUAL and performs a summary output of the qualitative data on the basis of:

1. The Apollo module.
2. Test sequence obtained from biochemical or other tests.
3. Category of organisms.

The lunar inventory subsystem is made up of a single program, LINT, the lunar inventory program. This program accepts estimates of the bioburden on spacecraft or parts thereof which have reached the lunar surface (prepared by DAST or entered directly), the masses of the impacting or landing hardware, estimates of their final velocities, and impact or touchdown locations and dates. Based on these data the densities of organisms delivered by these spacecraft or parts thereof, as well as the probabilities of future sample contamination, are calculated as functions of time and lunar coordinates. The total lunar bioburden from all hardware sources is also estimated as a function of time. A sample output is as follows:

	Longitude	Latitude		
AT COORDINATES	-0.000	-0.000	DATE	100.00*
DENSITY OF VIABLE VEGETATIVE MICROBES IS LESS THAN 4.47549E-05 PER SQUARE METER				
DENSITY OF VIABLE SPOREFORMER MICROBES IS LESS THAN 5.93078E-05 PER SQUARE METER				
PROBABILITY OF CONTAMINATION OF A ONE SQUARE METER SAMPLE				
	BY VEGETATIVE MICROBES		4.47539E-05	
	BY SPOREFORMER MICROBES		5.93060E-05	
	BY ANY VIABLE MICROBE		1.04057E-04	
TOTAL BURDEN ON LUNAR SURFACE AS OF DATE				
	VEGETATIVE		1.211E+08	
	SPOREFORMER		1.453E+08	

* Number of days after January 1, 1969

Subtopics and References

Lunar Information System Design; 6:9, 7:4, 8:5, 9:16, 10:13, 12:13, 18:69,
125, 126, 127

Users Manual; 77, 126

VII.B

Models

Two models for estimating the bioburden of a spacecraft at launch were used in this Lunar Information System, both for determining sampling requirements and for computing hardware bioburdens from a limited number of samples at desired confidence levels. These were discussed in Section VI. Two other major analytical efforts not specifically discussed elsewhere in this document are associated with the Lunar Information System.

LINT is essentially the coding of one of these models which:

1. Estimates the lunar density of terrestrial organisms deposited as a function of lunar coordinates and time.
2. Estimates the probability that a lunar sample will contain one or more terrestrial organisms previously deposited by missions, again, as a function of location and time.

The study which provided the model and hence the code for LINT consisted of five parts: initial burdens (at launch), burden change in cislunar space, dissemination mechanisms at the lunar surface, bioburden changes on the lunar surface, and the probability of sample contamination.

In general, estimates of initial (at launch) burdens of automated lunar capsules were both provided by NASA and computed using general environmental data. In addition, based on the general form of the model used for the prediction, one would not expect deviations from these estimates. Based on spacecraft and environmental data, the initial burdens were decomposed into "categories," including sporeforming organisms, vegetative organisms, exposed organisms, and interior organisms.

The effects of various physical phenomena in cislunar space on each category of organism was assessed. Among the phenomena considered were space capsule temperatures, vacuum, solar ultraviolet radiation, solar ionizing radiation, magnetosphere protection, and "near-earth" radiation belts. The general conclusion reached was that, in no case, did the burden at impact exceed 30% of the burden at launch.

For hard impacting spacecraft, two means of distributing a lunar probe's bioburden about the lunar surface were used: transport in crater ejecta and transport on space probe fragments. Other types of transport mechanisms were considered and generally discounted as being unimportant or yielding results somewhere between the "extremes" provided by the two mechanisms used.

In the examination of the transport mode when all microorganisms are attached to parts of the spacecraft, the assumption was that spacecraft break-up is explosive at the known hard impact velocities. This situation was analyzed to provide both particle velocity and particle range spectrums. With the assumption of a uniform spatial bioburden on the spacecraft, a lunar bioburden density was then obtained.

In the case where it was assumed that all microorganisms were contained in crater debris, calculations were based upon the work of Gault, Shoemaker and Moore. First, the mass of soil excavated was determined as a function of impact energy. The dispersal of this mass was then analyzed; the results were graphs of organism density on the lunar surface as a function of distance from impact site. Finally, the long term time dependence of this burden was analyzed.

For each of the two dispersal mechanisms, the probability of sample contamination was calculated per square meter of surface taken.

The conclusions drawn were of a "conservative" nature. These were:

1. Fewer than 30% of the microorganisms residing on a typical U. S. lunar probe at launch time survive transit to the moon. The thermal kill of organisms during the typical 34-80 hour transit times can be neglected.
2. Seven or eight months after touchdown, the contaminated area around the landing point of a typical U. S. mission that has made a soft landing should be confined within a conservative radius of 100 meters.
3. Organisms remaining on fragments of a typical U. S. lunar probe that has made a hard impact on the moon should be confined almost entirely within a conservative radius of 50-60 kilometers about the impact point. These may remain viable for indefinite periods of time.

4. Organisms carried by the crater material formed in the hard impact of a typical U. S. lunar probe may be deposited over the entire surface of the moon. Seven to eight months after impact, however, the contamination of the lunar surface from this dispersal mechanism should be negligible.
5. At 240-260 kilometers from the site of a hard impact of a typical U. S. lunar probe, the assumption of uniform deposition of the probe's bioburden over the entire lunar surface becomes a conservative assumption.

The other major model used in the Lunar Information System is the computerized identification system which simulates the PHS identification of colonies selected from spacecraft on the basis of biochemical and other tests of their properties. Inasmuch as the results of these tests were available in QUAL, Sandia was asked to investigate the possibility of having the computers used by the Lunar Information System perform the identification that had been done by hand by the Public Health Service. The PHS identification system consists of two essentially different parts. The first part is a flow diagram which uses the results of five tests to enter the second portion of the system. This second part consists of 13 schemes which permit an identification to be made on the basis of certain tests selected by the outcomes of the 5 tests used in the first part. The schemes used in the second part vary from complicated keys relating a number of tests and organisms to simple keys which admit an identification based only upon the five tests used in the first part of the scheme. The computer simulation of the first part was quite straightforward.

In general, the schemes of the second part of the system consisted of a matrix relating (through test results) tests and the identity of the organisms associated with the scheme. Hence, in the simplest case, given a colony known to belong to some scheme, the outcomes of the required tests could be read from QUAL as a Test Vector and compared with the scheme matrix until a match was obtained which would provide the desired identification. Unfortunately, it developed that frequently an exact match did not occur between the unknown Test Vector and results associated with any organism category in the scheme to which it presumably belonged. As a result the "best possible" match (or identification) was considered. This could occur in a number of ways. An identification could occur with an exact match except for a single test result which disagreed with that anticipated for the organism's category.

It might happen that there was a single disagreement with the results to be anticipated for two distinct organism categories or three or more organism categories. Similarly, there could be disagreement on two tests for one or more organism categories. As a result of these considerations, the computerized system indicated the level at which an identification was made in accordance with the following table.

<u>No. of Test Disagreements</u>	<u>No. of Categories</u>	<u>Computer Statement of RESULT</u>
0		EXACT IDENT
1	1	ONE DISAGREEMENT
1	2	2 SINGLE DISAGREEMENTS
1	3	3 SINGLE DISAGREEMENTS
2	1	2 DISAGREEMENTS
Other		NO ID POSSIBLE

Obviously, these options imply that multiple identifications will occur, e.g., 3 SINGLE DISAGREEMENTS, with three organism categories being equally possible using these selection rules. The level at which an identification was made could be varied at will. The above was selected as representing PHS practice with the Apollo data.

In accordance with the above considerations, a program was written which simulated the PHS identification system. To determine the feasibility of this approach, the program was exercised with the Apollo 11 data as obtained from QUAL.

After the feasibility demonstration with the Apollo 11 data, some of the identification scheme was changed and the program was incorporated into QUAL. In its new form it yielded better than 93% agreement with PHS identifications on Apollo missions 12 and 13. Commencing with Apollo 14, the identification of colonies was done entirely by this program in QUAL.

A much more general (and efficient) program has been written and checked out. This program will accept almost any identification system as input, and since the system itself is input to the program, it is very easy to change from one system to another.

Subtopics and References

Lunar Inventory Model; 5:16, 6:10, 7:6, 9:16, 90

Computerized Identification; 15:35, 16:16, 17:58, 19:49, 21:67, 38, 39

VII.C

Consulting

Sandia has been active in consulting in problems connected with lunar exploration. These consulting activities have been with not only NASA personnel, but have also involved other organizations in the planetary quarantine community such as the Public Health Service. Without attempting to discuss in detail the many exchanges that have taken place, we shall only mention some of the more interesting problems.

The consulting in lunar programs can be divided into two classes. The first of these is a general area of program planning of the planetary quarantine activities. Among the assistance given for these types of problems are:

1. Planning of sampling programs - In this area, Sandia developed models for both the qualitative and quantitative aspects of the sampling programs for Apollo modules (Section VI).
2. Computer facilities - Sandia has advised and evaluated the various proposals for computers and their utilization.
3. Data handling - Sandia has developed models and applied other models and techniques in the analysis of data for other organizations.

The other main area of consulting in lunar programs is connected with specific mission planning problems. Some of these have been:

1. SLA, IU, SIVB Impact - The landing coordinates of the SLA, IU, and SIVB are not usually known. The question arose concerning the contamination of future lunar samples due to the terrestrial organisms from this impact. We determined that the probability of such contamination is less than 10^{-6} for the subsequent Apollo flight.
2. LM ascent stage impact - A problem arose concerning the approval of the impact of the LM ascent stage after docking in lunar orbit. This question centered around the problem of organic contamination. We found that the probability of contaminating a one square meter

sample on the subsequent Apollo flight ranged from 10^{-5} at 10 meters from the impact to 10^{-7} at 100 meters.

3. Urine release - A question arose concerning the venting of urine from the Apollo Lunar Module onto the lunar surface. It was found that the original plan would lead to significant levels of organic contamination in sampling areas. Appropriate suggestions were made on the modification of the hardware so that the contamination level would be acceptable.

Subtopics and References

SLA, IU, SIVB Contamination Calculations; 11:8

Quantitative Sampling; 74, 78

Qualitative Sampling; 75

Section VIII

Contamination Control

Efforts in this area have been on behalf of both the AEC and NASA. Activities have arisen from direct needs in both agencies, and the knowledge thus gained has been made widely available in the private sector.

VIII.A

Contamination Control Principles

The document, Contamination Control Principles, presents a model which relates contaminant types and sources to specific environments which could be exposed to these contaminants. This model enables the planner as well as the practitioner to organize contamination control efforts in a manner which considers all facets of the problem and provides a logical sequence of activity to attain the stated objective.

This basic document provides an overview of the field that is equally as informative to the neophyte as to the manager under whose direction the contamination control program will be implemented. In addition to describing the different types of contaminants, it provides information on the means for removing or controlling contamination and for monitoring to determine what contamination levels exist.

This document was prepared by Sandia under NASA Contract No. W-12324.

Subtopics and References

Contamination Control Principles; 4:13, 5:17, 6:15, 7:7, 88

VIII.B

Contamination Control Handbook

The need for a handbook to promote a better understanding and proper application of contamination control in aerospace and allied industries was recognized by a panel of NASA contamination control specialists. As a result, a Contamination Control Handbook was prepared by Sandia for NASA under NASA Contract No. H-13245A. The handbook assembled in one document reliable technical information and data which had been developed by Sandia or which was not readily available from any one source.

In its nearly 400 pages, 99 tables and 62 figures, the handbook emphasizes the systems approach to contamination control and presents information on the following subjects as they relate to contamination control:

- Philosophy and Modeling
- Product Design
- Surface Contamination
- Gas and Liquid Contamination
- Airborne Contamination
- Microbial Contamination
- Radiation
- Packaging
- Handling and Storage
- Personnel

More than 3000 copies of the handbook are now being used by many diverse industries throughout the United States and in other parts of the world.

Subtopics and References

Contamination Control Handbook; 4:13, 5:17, 6:16, 7:7, 8:17, 9:27, 10:3, 11:3, 12:3, 54

VIII.C

Federal Standard 209a

Under authority delegated by the General Services Administration, Sandia (through the Atomic Energy Commission) accepted the responsibility to prepare and maintain Federal Standard 209, "Clean Room and Work Station Requirements, Controlled Environment."

This document establishes and defines standard classes of air cleanliness and provides information needed to conform to these class levels. The use of this standard is mandatory on all federal agencies.

At the present time, the standard is being revised to become 209b and is being coordinated with 12 federal agencies and 14 industrial concerns.

Subtopics and References

Federal Standard 209; 14:44, 17:66, 20:67, 21:75, 22:43, 23:51, 56

VIII.D

Contamination Control Consulting

Although contamination control consulting has played a minor role in the total activities of the Department, it has made a significant contribution to the accomplishments.

Many of the requests for information and assistance have originated with Sandia engineers and are related to weapon products being manufactured by AEC Integrated Contractors or Sandia suppliers. Other sources of requests for consultation are listed below:

AEC - Headquarters, LASL, Oakridge

NASA - Headquarters, Space Centers, Contractors

Other - Federal Agencies, Hospitals, Industry

While much of the assistance requested concerns clean room design and requirements, a number of other areas are covered, such as sources and capability of equipment, specifications and standards, cleaning and monitoring methods, check-out procedures, and personnel regulations.

One product of consulting has been the saving of time and money through our ability to provide immediate and accurate information, thereby eliminating the need for lengthy information searches. Perhaps the most significant contributions, however, have been in the following two areas:

1. Recommending a system approach to the solution of contamination control problems by recognition of all factors involved in a given situation, rather than just those immediate and obvious factors.
2. Recommending correct and adequate but not excessive equipment and procedures, thereby providing acceptable quality at the lowest reasonable cost.

Subtopics and References

Consultation; 1:11, 58, 59

Notable Activities and Awards

1. H. D. Sivinski, Chairman, AEC/NASA Symposium on Contamination Control, Theme - "Current and Advanced Concepts in Instrumentation and Automation," Albuquerque, New Mexico, September 12-14, 1967.
2. H. D. Sivinski, Program Chairman and Moderator, 9th Annual Symposium of the New Mexico Section, ASME, and the University of New Mexico College of Engineering, Theme - "Responsible Technology: A Mandate for Engineering," Albuquerque, New Mexico, November 15-16, 1968.
3. W. J. Whitfield, "Individual Scientific Technical Achievement Award," American Association for Contamination Control, New York, May 1969.
4. H. D. Sivinski, Moderator, Symposium on "Clean Room Technology in Surgery Suites," Sponsored by the National Aeronautics and Space Administration and the Midwest Research Institute, Kennedy Space Center, Florida, May 21-22, 1971.
5. W. J. Whitfield, "Holly Medal," American Society of Mechanical Engineers, Los Angeles, California, November 1969.
6. W. J. Whitfield, Honorary Doctor of Science Degree, Hardin-Simmons University, Abilene, Texas, May 1970.
7. W. J. Whitfield, "Research Achievement Citation," European Contamination Control Foundation, Stuttgart, Germany, June 1970.
8. J. P. Brannen, Visiting Scientist Lecturer, Society for Industrial and Applied Mathematics, 1971-1972.
9. D. M. Garst, National Secretary, American Association for Contamination Control, 1971-1973.
10. J. P. Brannen, Program Committee, Biomathematics Symposium, Society for Industrial and Applied Mathematics, 1969.
11. J. P. Brannen, Conference Staff Member, Applications of Operations Research to Water Pollution Control, New Mexico State Technical Services Symposium, LASL, 1968.
12. C. A. Trauth, Jr., Fellow of the Royal Society of Health, 1972.

Patent Activity

Patents Issued:

1. U. S. Patent No. 3,273,323, covering a Laminar Airflow Hood Apparatus, issued September 20, 1966 to W. J. Whitfield.

Patent Disclosures:

1. Therapeutic and Nontherapeutic Applications of Thermoradiation, Ralph E. Trujillo and Virgil L. Dugan.
2. Biological Inactivation by Various Dilute Formaldehyde Solutions, Ralph E. Trujillo.
3. Preparation and Properties of Formaldehyde Gels, Ralph E. Trujillo.
4. A New Formaldehyde Base Disinfectant, Ralph E. Trujillo.
5. Thermoradiation Sterilization, Marcel C. Reynolds.
6. Refuse Bag Retainer, Marcel C. Reynolds.
7. Vacuum Probe Sampler, W. J. Whitfield and Virgil L. Dugan.
8. Precision Dilution System, W. J. Whitfield, W. E. Neitzel and J. C. Mashburn.
9. Optical Particle Counter Chamber, W. J. Whitfield, R. C. Marsh and F. W. Oswalt.
10. Optics System for Particle Converter, W. J. Whitfield.
11. Automatic, Instantaneous Monitor for an Aerosol, Virgil L. Dugan.
12. Device to Measure Adhesion Forces, Marvin E. Morris.
13. Acoustic Dust-Feeder, Marvin E. Morris.

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Interdisciplinary Communications

In addition to the numerous journal articles, technical reports and presentations describing the work of the Planetary Quarantine Department, the technical news media has further publicized this work in the following instances:

Thermoradiation

Sandia Science News, Vol. 4, No. 4, September 1969

Chemical & Engineering News, p. 39, September 29, 1969

Chemical Week, October 11, 1969

Aviation Week, p. 8, November 17, 1969

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Sandia Science News, Vol. 6, No. 3, September 1971

Contamination Control Handbook

NASA Tech Brief, October 1968

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J. A. Hornbeck - 1
C. Winter - 100
W. J. Howard - 1000
D. B. Shuster - 1200
C. B. McCampbell - 1310
W. A. Gardner - 1500
H. E. Lenander - 1600
T. M. Burford - 1700
A. A. Lieber - 1710
G. J. Simmons - 1720
D. P. Peterson - 1724
R. G. Clem - 1730
H. D. Sivinski - 1740 (35)
J. M. Wiesen - 1750
B. H. Van Domelen - 1913
A. M. Clogston - 5000
L. C. Hebel - 5200
J. V. Walker - 5220
R. M. Jefferson - 5221
J. E. McDonald - 5300
L. M. Berry - 5500
R. W. Henderson - 7000
L. S. Ostrander - 8232
G. A. Fowler - 9000
J. H. Scott - 9200
C. F. Bild - 9300
L. A. Hopkins, Jr. - 9400
D. W. Ballard - 9461
W. K. Cox - 3141-1 (15)
Tech. Info. I - 3151 (2)
(For AEC/TIC)