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MICROBIOLOGICAL ASPECTS OF THE PLANETARY QUARANTINE PROGRAM FOR VOYAGER

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MICROBIOLOGICAL ASPECTS OF THE PLANETARY QUARANTINE PROGRAM FOR VOYAGER

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SECTION 1

INTRODUCTION

The prime goal of the Planetary Quarantine Subtask, under the Voyager Phase 1A Task C Study, is to show the effect of Planetary Quarantine requirements on the Voyager mission and its elements.

Figure 1-1 is a simplified work flow diagram showing the major Planetary Quarantine subtasks and their interrelationships. Activities performed under this contract are being documented in bimonthly progress reports and a separate series of technical reports and memoranda. The present report presents the results of the activities under the Biological Studies subtask. Section 2 of this report briefly summarizes these results. In Section 3, a catalog of the biological loading associated with various levels of manufacturing contamination control is presented. Section 4 presents data as to the effects of interplanetary environmental factors on the viability of microorganisms. The conclusions and recommendations drawn are presented in Section 5. Finally, Section 6 presents a detailed listing of the references used herein.

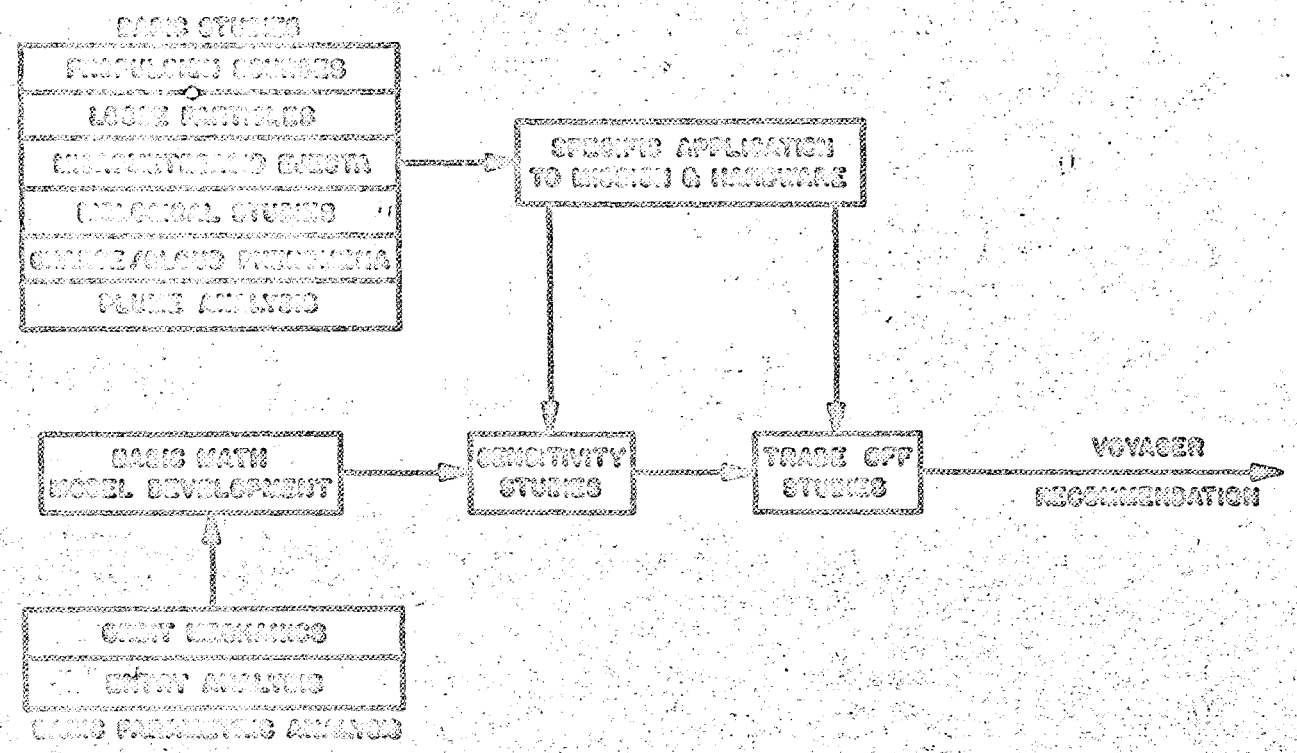

Figure 1-1. Planetary Quarantine Task, Simplified Work Flow Diagram

SECTION 2

SUMMARY

The activities in the Biological Studies subtask of the Planetary Quarantine Study consisted primarily of the following:

a. The generation of a Biological Burden Catalog (see Tables 3-3, 3-4 and 3-5) from a survey of current and completed studies. The catalog depicted herein displays the biological burden level for typical spacecraft materials and structures as a result of the biological contamination control imposed during production.

   In the preparation of the burden catalog, it became apparent that information, although sparse, is available on the bioload of spacecraft and spacecraft hardware. From this information, cautious predictions may be made to assess the probable ranges of numbers of microorganisms present on or in such hardware.

b. The survey, analysis, and extrapolation of data available on the potential lethal effects of the interplanetary environment. The principal environmental factors studied were:

   1. Ultraviolet radiation in the space and planetary environment

   2. The thermal environment of interplanetary space

   In addition, several other factors were considered in the study and are reported herein. These factors, which have been found to be more poorly defined experimentally than the principal ones listed above, are:

   1. Cosmic radiation

   2. Vacuum

   3. Vibration

   Table 2-1 depicts the environmental factors and summarizes their respective effects on the viability of microorganisms.

Table 2-1. Environmental Factors Which Could Effect the Viability of Microbial Contaminants on A Spacecraft

Environmental Factor	Level or Range	Effect
Temperature	Cold - below 0°C	Preservation effect
	Intermediate 0°C - 60°C	Could aid growth if nutrients present
	Extraplanetary flight - hot > 60°C	If greater than growth temperature can reduce number of viable organisms dependent upon length of exposure
	Planetary entry - ultrahigh	Sterilize surfaces
Vacuum	To 5 microns pressure ultrahigh	Preservative effect, especially if applied to a frozen preparation
Desiccation		Generally preservative, especially if combined with vacuum
Gaseous Conditionants		Reduced O_2 content (generally not detrimental with vacuum)
Radiation		
Strongly ionizing types particulate Van Allen Belt Gamma X-ray		Capable of exerting significant kill effect on freely exposed organisms on surfaces; dependent upon total exposure
Solar ultraviolet Solar - soft x-ray		Capable of reducing the number of freely exposed viable organisms
Cosmic rays	10^0 - 10^1 rad/year 10^2 - 10^6 rad/year through 10^{-2} g/cm^2	Will not eliminate microbes on exposed surfaces

SECTION 3

MICROBIOLOGICAL BURDEN OF SPACECRAFT HARDWARE

3.1 BACKGROUND

The current Planetary Quarantine Study task requires information with respect to the density or level of microbiological contamination that might be anticipated on a spacecraft at the termination of manufacture. The variations in the biological burden as a result of the microbiological contamination controls which might be imposed on standard manufacturing processes and practices, as well as what might be expected under normal conditions, have recently been the objective of several studies (References 45 through 54). The survey and evaluation undertaken as part of the effort reported herein has attempted to combine all of the available and useful information into a summary catalog.

3.2 THE BIOBURDEN CATALOG

The Bioburden Catalog is presented in Tables 3-3, 3-4 and 3-5. Most of the data are the results of direct assay of equipment and hardware, performed by the groups identified in the references. It should be noted that this catalog is hence a compilation of many measurements and some "guesstimates." Some discrepancies will be found in comparing levels under the various conditions noted in the tables. These, for the most part, are due to the fact (i) that the various groups employed various assay techniques to obtain their respective results and (ii) that any assay is a result of the bioload at any one stage in processing or manufacture, which is in turn dependent upon the effects of the many specific load-reduction or load-increase factors.

The various bioloads reported for each case are, of course, dependent upon the accuracy of the assay technique employed. The usual accuracies associated with the various assay techniques which were used are summarized in Table 3-1. One approach to the evaluation of the variation in the total bioload attributed to the various factors is presented in Table 3-2.

Generally, estimation of the various types of microbiological contaminants has been restricted to total aerobic mesophilic counts, with spore and anaerobic counts being performed in only a few studies. However, it appears that in their cases where direct comparisons have been made that the spore population represents about 10 percent of the total count. This may serve as a value for estimation until additional data becomes available.

Table 3-1. Summary of the Accuracies of Various Microbiological Assay

Assay Technique	Precision	Recoveries (percent)	References
Swab	Poor	40 to 100	Angelotti (1958), Bond et al (1963)
Rinse (Direct flush only)	Fair	80	Buchbinder (1947) Angelotti (1958)
Rinse - Mechanical shake*	Fair	50 to 100	General Electric (1967) (to be published)
Rinse - Ultrasonic agitation**	Best	95 to 100	Puleo et al (1967)
Rodac	Good	40 to 50	Bond et al (1963), Angelotti (1964)
Rinse/Filtration/Culture	Good	90 to 100	Millipore (1965)
Size reduction techniques			
Experimental samples	Fair	80 to 100	Puleo (1966)
Solid materials and components	Poor	1 to 10	McNall (1965)

*This technique is being employed in the General Electric Combustion Lethality Experiment [illegible]. The recovery percentages shown herein are based on preliminary [illegible] program. These tests include the recovery of organisms from large surface areas ([illegible] square inches).

**The data for this technique was obtained with the use of stainless steel strips and some electronic components.

Table 3-2. Variation of Total Burden Attributable to Contaminating Factors

Parameter	Variation Range	Percent Variation of Total Burden
1 Internal burden	± order of magnitude	30.5
2 Fallout	30 to 120 org/ft²/day	53.5
3 Electrostatic effect	1 to 10 [illegible]	32.3
4 Die-off	30 to 99 percent	64

Conditions: Each parameter varied, holding others constant

No [illegible] test or clean room

Source: Reference [illegible], page [illegible]

In order to provide as much information on bioburden as is possible, the data available from studies which employed the sterile metal strip assessment technique in the various classes of controlled environment facilities has been summarized and is presented in Part III of the catalog (Table 3-5). Although the primary interest was placed on hardware and simulated hardware (fallout, handling, etc.), there is other data that may be useful in evaluating the bioburden. These include results from sampling the air, clothing, rodac plate, and swab sampling of the clean room area itself and the tools.

For more specific information, the reader is advised to consult the specific publications referred to in the catalog.

3.3. FACTORS AFFECTING BIOBURDEN*

Items and conditions that can be incorporated into the Bioburden are (1) internal burden comprised of contaminants in raw materials and parts; (2) burden added or subtracted in handling, assembly, and decontamination process; and (3) occluded burden. Occluded burden is that contamination added to the system when two surfaces are joined.

In a "bioclean" facility, the burden is dependent upon the number of persons in contact with the spacecraft. With proper clothing, and controls, etc., the burden may be reduced. Conservative estimates are a reduction of two orders of magnitude. Plastic material may multiply the burden many-fold due to the effects of electrostatic charge. Under artificial conditions, the presence of plastic materials may increase the burden by a factor as high as 18 times that otherwise present. A conservative value is a factor of 5.

*Based on work reported in Reference 50.

The sensitivity of the bioburden to several contaminating factors has been discussed by AVCO (Reference 50) and summarized in Table 3-3. These factors include:

a. Microbial fallout existing in air-conditioned aerospace assembly and test facilities is on the order of 30 to 50 organisms per square inch per day.

b. Microorganisms (under nonnutritive condition) tend to die off in components. Examples of die-off rates are as follows:

   1 percent per day
   30 percent per month
   99 percent per year

c. Contamination factors consist of fallout on surfaces in a normal facility (32 to 128 organisms per square inch per day) and in a bioclean facility (0.32 to 1.28 organisms per square inch per day). Handling of component parts and materials in a normal facility might be about 1000 organisms per square inch of contact surface. In a "bioclean" facility it might be about 10 organisms per square inch of contact surface. An electrostatic concentration factor of between 1 and 10 might exist, depending on the specific conditions.

d. The consensus values for decontamination factors are, for 4 days of treatment with ethylene oxide (12 percent EtO, 88 percent Freon-12 mixture), a reduction of 10^{-6} is achieved. Ethylene oxide will not reach the internal sealed component parts, and any decontamination effect would be limited to surfaces that the ethylene oxide could reach. Flight-acceptable dry heat test at 125 to 135°C for 1 to 2 days results in a reduction of 10^{-12}.

   As a result of sterility tests performed on several hundred electronic components (capacitors, diodes, resistors, etc.), the presence of microbial contaminants has been demonstrated in only about 10 percent (References 36, 41, and 42). This is not surprising, since many electronic parts receive some heat or chemical treatment during the course of their manufacture. However, one should be cautioned that the other 90 percent may not be sterile but only that viable microorganisms could not be detected.

Table 3-3. Bioburden Catalog, Part I – Typical Spacecraft Components

Material, Component, Structure \ Manufacturing Environment	Level of Microbial Contamination *						
		Controlled Environment Facilities					
	Normal Manufacture	Class 250,000	Class 100,000	Class 100	"Bioclean" Facility	Reference	Comments
	**						
Cable assembly (various connectors)	None/4.7 x 10^3					(45)(46)	Assay
Pneumatic valves	<1.0 x 10^3/1.2 x 10^4					(45)(46)	Assay
Electrolitic adapter						(45)(46)	Assay
Internal surface			6.0 x 10^0/6.0 x 10^1				
Interior surface			1.69 x 10^0/1.4 x 10^3				
Component surface			7.52 x 10^0/12.3 x 10^2				
Component surface			6.63 x 10^3				
Electromechanical component (accumulator guarantee)				None/4.2 x 10^3		(45)(46)	Surfaces exposed aseptically and assayed directly
Pneumatic components						(45)(46)	Residual level depends on cleaning procedure Assay
Pressure gauges	None/2.6 x 10^4 (total count) None/3 x 10^3 (spore count)						
Valves	6.0 x 10^0/4.0 x 10^1 (total) 2.0 x 10^0/2.2 x 10^3 (spore)						
Pressure regulators	1.3 x 10^0/2.0 x 10^3 (total) None/3 x 10^3 (spores)						
Tubing	3 x 10^3/3 x 10^3 (total)						
Protective pods							Residual level depends on time in laminar flow and position as regards personnel, as well as direction of air flow, i.e., vertical and horizontal
Optimum-case density of a small planetary lander							
Exterior surface					5.0 x 10^0/2.8 x 10^1	(48)	
Interior surface					4.0 x 10^0/1.6 x 10^2		
Honeycomb aluminum							
Exterior surface	<300						
Black box (roll sensor digital processor) (8 x 6 x 8 in.)		1 x 10^3				(45)	2.7 x 10^4 (total) Assay
Panel – Int to Encap.		2.0 x 10^3					
Ext to Encap.		1.4 x 10^3/2.0 x 10^3					
Sheet metal		4.0 x 10^3/8.0 x 10^3					
Connector		3.1 x 10^3/3.2 x 10^3					
Screws		3.0 x 10^3					
Wire harness		3.0 x 10^3					
Screw hole		6.1 x 10^3				(49)	
Rocket motor propellant coating	8.4 x 10^2/1.5 x 10^3						
Typical surfaces:							
Horizontal component surface		2.8 x 10^3	2.3 x 10^3/4.0 x 10^3	1.1 x 10^2	8.0 x 10^1	(45)(46)	Assay
Vertical component surface		4.9 x 10^2	7.0 x 10^1/4.0 x 10^3	7.0 x 10^1	7.0 x 10^1	(45)(46)	Assay
Structural surface		3.6 x 10^3	2.5 x 10^3/3.0 x 10^3	5.9 x 10^2	1.4 x 10^2	(45)(46)	Assay
Micro-electronic modules 1 x 1 1/2 x 7/16 in.	3.0 x 10^1/1.4 x 10^3			2.6 x 10^4	6.5 x 10^2	(45)(46)	Assay
Electrical harness	3.9 x 10^3 (total) 2.2 x 10^3 (spores)			≤2.6 x 10^3	<2.6 x 10^3	(45)(46)	Assay
Balsa wood	1.0 x 10^0/1 x 10^2 in.^{3}					(50)	
Capacitor	1.0 x 10^1/1.0 x 10^3			0/1.0 x 10^1		(50)	
Coaxial cable	0/1.0 x 10^2/ft			0/1.0 x 10^0		(50)	
Connector	0/1.0 x 10			0		(50)	
Diaphragm	1.0 x 10^3/gm			1.0 x 10^1/gm		(50)	
Diaphragm (nylon)	0/2.0 x 10^3/ft			0/2.0 x 10^0/ft		(50)	
Foam	1/ml			0		(50)	
Inductor	1.0 x 10^3/1.0 x 10^3			1.0 x 10^1/1.0 x 10^2		(50)	
Microwave	0/1.0 x 10^1			0		(50)	
Optical system	1.0 x 10^1/1.0 x 10^3			0/1.0 x 10^4		(50)	
Relay	1.0 x 10^3/1.0 x 10^3			1.0 x 10^0/1.0 x 10^1		(50)	
Resistor	0/1.0 x 10^1			0		(50)	
Silicone integrated circuit	0/1.0 x 10^1			0		(50)	
Silicone	1/ml					(50)	
Transformer	1.0 x 10^4/1.0 x 10^5			1.0 x 10^2/1.0 x 10^3		(50)	
Structure No. 1*				21,174	563	(51)(AIMP)	Assay
Structure No. 3*				22,330	230	(51)	Assay
Structure No. 4*				2,125	90	(51)	Assay
Structure No. 5*				1,209	219	(51)	Assay
Cocktail Section A* (Interior surfaces of module frames including walls, conduits and electrical circuit boards and containing electrical components)				prototype – 412 flight – 1152	prototype – 39 flight – 36	(51)	Assay
Cocktail Section B* (Cocktail surfaces contacted by module frames, containing exposed surfaces on the inside of module frames)				prototype – 3368 flight – 224	prototype – 36 flight – 23	(51)	Assay
Cocktail Section C* (Inner surfaces of spacecraft cocktail by protective cover)				prototype – 1905 flight – 228	prototype – 29 flight – 33	(51)	Assay
Cocktail Section D* (Exterior surfaces and volumes of the spacecraft)				prototype – 1225 flight – 307	prototype – 53 flight – 39	(51)	Assay
Cocktail Section E* (Exterior surfaces of spacecraft)				prototype – 2034 flight – 162	prototype – 18 flight – 28	(51)	Assay

*See page 3-6 for definitions of AIMP structures and cocktail sections.

**All units in number of organisms (low/high) per square foot, unless otherwise indicated

Table 8-3. (Cont'd)

Definitions of AIMP Structures and Occluded Sections (Reference 51)		
Structure No. 1	-	C-frame base and platform mating surface
	-	Lower cone support ring and platform mating surface
	-	C-frame
	-	Antenna cup brackets (8)
	-	Stiffeners (26)
	-	Despin-disconnect brackets (6)
Structure No. 2	-	Yo-yo disconnect brackets (2)
	-	Struts (8) and center tube mating surface
Structure No. 3	-	Platform lower surface
	-	Paddle-support brackets (4) and platform mating surface
	-	Struts (4) and paddle-support brackets mating surface
	-	Boom-support bracket and platform mating surface
	-	Boom brackets (4) and boom-support bracket mating surface
	-	Platform-support brackets and platform mating surface
	-	Struts and platform-support mating surfaces
	-	Struts (8) and boom-support bracket mating surfaces
	-	Paddle hinge (4) and paddle-bracket mating surface
	-	Paddle-arm restraint (4) and mating surface
	-	Unofficial connector bracket (2) and platform mating surface
	-	Solar-cell damage experiment connector bracket (2) and mating surface
Structure No. 4	-	Paddle spring brackets (4) and mating surfaces
	-	Fourth-stage flyaway boxes (4) and mating surfaces
	-	Back of fourth-stage spring housing and mating surface
	-	Back of fourth-stage microwave assembly and mating surface
	-	Pitot arms (4) and platform mating surfaces
	-	Black damp radiation box (2) and platform mating surface
	-	Center tube bracket

Table 8-3. (Cont'd)

Definitions of AIMP Structures and Occluded Sections (Reference 51) (Cont)		
Structure No. 4 (Cont)	-	Interior of Goddard boom (large sect)
	-	Interior of Ames boom (large sect)
	-	Interior of Goddard boom (small sect)
	-	Interior of Ames boom (small sect)
	-	Goddard boom cable (outer surface of bundle)
	-	Ames boom cable (outer surface of bundle)
	-	Flungate boom connector plate (Goddard)
	-	Flungate boom connector plate (Ames)
Structure No. 5	-	Flungate boom gold insert (Goddard)
	-	Flungate boom gold insert (Ames)
	-	Flungate boom housing interior (Goddard)
	-	Flungate boom housing interior (Ames)
Occluded Section A	-	GSFC flungate A/D electronics
	-	GSFC flungate electronics
	-	Optical aspect computer
	-	Optical aspect sensor
	-	Prime computer
	-	Programmer No. 1 (multivibrator)
	-	Solar-array regulator
	-	LEF plasma probe
	-	Antenna splash
	-	Telemetry encoder
	-	Range and range-rate No. 2
	-	Range and range-rate No. 3
	-	University of California ion chamber
	-	Ames signal processor
	-	University of Iowa [illegible] Time-to-and-[illegible] [illegible]
		[illegible] number No. 2 (IV stage [illegible])

Definitions of AIMP Structu		
Occluded Section B	-	C-frame
	-	Platform
	-	Top of m
	-	Front fac
	-	Inner sur
	-	Struts, C
	-	Center tu
	-	Back of
	-	Antenna
	-	Antenna
	-	Yo-yo de
Occluded Section D	-	Center tu
	-	Sun shiel
	-	Sun shiel
	-	Fourth-s
	-	Fourth-s
	-	Fourth-s
	-	Battery
	-	Battery
	-	Spring s
	-	Third-st
	-	Lower c
	-	Center t
	-	Platform
Occluded Section E		

...ections (Reference 51)
...ing surface
...tform mating surface
...g surface
... platform mating surface
...ator's mating surface
...mm mating surface
...ment bracket mating surface
...platform mating surface
...ting surfaces
...chet mating surfaces
...chet mating surface
...ting surface
...and platform mating surface
...connector bracket (A) and
...ating surfaces
...nal mating surfaces
...ting and mating surface
...h assembly and mating surface
...ting surfaces
... platform mating surface

Table 3-3. (Cont'd)

Definitions of AIMP Structures and Occluded Sections (Reference 51) (Cont)		
Structure No. 4 (Cont)	-	Interior of Goddard boom (large sect)
	-	Interior of Ames boom (large sect)
	-	Interior of Goddard boom (small sect)
	-	Interior of Ames boom (small sect)
	-	Goddard boom cable (outer surface of bundle)
	-	Ames boom cable (outer surface of bundle)
	-	Fluxgate boom connector plate (Goddard)
	-	Fluxgate boom connector plate (Ames)
Structure No. 5	-	Fluxgate boom gold insert (Goddard)
	-	Fluxgate boom gold insert (Ames)
	-	Fluxgate boom housing interior (Goddard)
	-	Fluxgate boom housing interior (Ames)
Occluded Section A	-	GSFC fluxgate A/D electronics
	-	GSFC fluxgate electronics
	-	Optical aspect computer
	-	Optical aspect sensor
	-	Prime computer
	-	Programmer No. 1 (multivoltage)
	-	Solar-energy regulator
	-	RMT plasma probe
	-	Antenna lighted
	-	Telemetry encoder
	-	Ranger and ranger-mate No. 2
	-	Ranger and ranger-mate No. 3
	-	University of California ion chamber
	-	Ames signal processor
	-	University of Iowa plasma wave and electron plasma
		Programmer No. 2 (27 stage sequencer)

Table 3-3. (Cont'd)

Definitions of AIMP Structures and Occluded Sections (Reference 51) (Cont)		
Occluded Section B	-	C-frame occluded by circuit modules
	-	Platform top covered by module frame
	-	Top of module stack
	-	Front face of stack
	-	Inner surface of cover
	-	Struts, C- frame to center tube
	-	Center tube from platform to cover
	-	Back of C-frame
	-	Antenna cups below cover
	-	Antenna supports
	-	Yo-yo despin connector bracket
Occluded Section D	-	Center tube interior
	-	Sun shield plate
	-	Sun shield support rod
	-	Fourth-stage spring assembly housing
	-	Fourth-stage microswitch assembly
	-	Fourth-stage flyaway connector box
	-	Battery bracket and connector assembly
	-	Battery
	-	Spring seat assembly
	-	Third-stage separation microswitch assembly
	-	Lower cone
	-	Center tube under lower cone
	-	Platform under lower cone
Occluded Section E		

2

Table 3-4. Bioburden Catalog, Part II - Typical Spacecraft

Manufacturing Process / Material Component Structure	Level of Microbial Contamination*						
		Controlled Environment Facilities					
	Normal Manufacture	Class 250,000	Class 100,000	Class 100	"Bioclean" Facility	Reference	Comments
Pilot Spacecraft** (Total)		8.20×10^3 (total) 7.4×10^6 (spores)	6.2×10^3/3.60×10^3 (total) none/4×10^4 (spores)	7.6×10^6 (total) $<1 \times 10^6$ (spores)	2.1×10^6 (total	(45) (46)	Calculations based on assay data
Pilot spacecraft (specific) Pneumatic valves (external) Pressure regulator (external) Stainless steel tubing (external) Propellant tank (external) Horizontal surfaces Vertical surfaces Honeycomb panel Structure Electronic modules Shock fix assembly Structure Electronic subsystem Horizontal surface Vertical surface		7.0×10^3 $<10^3$/1.8×10^3	$<10^3$/4×10^3 $<10^3$ $<10^3$/3.0×10^3 1.4×10^3 3.6×10^2 7.0×10^1 0/4.0×10^2 1.1×10^3/2.4×10^3 1.8×10^3/1.4×10^3 8.0×10^1/2.7×10^3 3.0×10^3 7.0×10^1	1.4×10^1/2.2×10^3		(45) (46) ***	All this information is for total organisms. Range depends on cleaning procedure. All are results of assay.
Biomedicine (solids)		1.29×10^6 (total)	8.7×10^4/1.61×10^6 (total)	2.0×10^6	4.5×10^4	(45) (46)	
[illegible] Component [illegible] Electrical Equipment Package Assembly [illegible] Mod. [illegible] Rec. Processor Clock board (pull) Tel. Pro. Computer Tel. Proc. Clock Tel. Processor Clock board (pull). Telemetry processor Poly. Nylon storage bag Metal connector Connector			1.0×10^3 7.0×10^1 none/6.0×10^3 1.0×10^3 1.4×10^3 none 1.4×10^3 7.0×10^1 none/6.0×10^3 1.0×10^3 2.0×10^3			(45) (46)	Results of microbiological assay as total counts
[illegible] Hyperconstitution Infrared Rad. Rec. Automatic pressure tubing system [illegible] Auxiliary load panel Thermal control shutter Thermal control shutter Auxiliary load panel Thermal controller Component bracket Chip [illegible] [illegible] [illegible] [illegible] Hyperconstitution Infrared Rad. SCIP Auto. uplink communications systems Clipped end of pin connection Clipped [illegible] connection [illegible] APCS [illegible] bundle [illegible] with finger sandpe [illegible]			1.0×10^3 2.0×10^3 4.7×10^2 4.8×10^3 3.0×10^2 4.0×10^2 $>10^3$ 3.0×10^3 4.1×10^3 6.1×10^3 3.0×10^2 3.0×10^3 2.0×10^2 1.4×10^2 $>10^3$ >0 6.4×10^2 1.0×10^3 3.0×10^2 2.0×10^3			(45) (46)	Results of microbiological assay as total counts
[illegible] [illegible] [illegible] Wiring and [illegible] [illegible] [illegible] Component [illegible] assembly Organic [illegible] cabinet Electrical cabling Component with electrical cabling All organic electrical cabling Thermal zone electrical cabling			none/6.0×10^3 7.0×10^3 1.1×10^3 1.1×10^3 1.0×10^3 7.0×10^1/4.00×10^3 7.0×10^3 6.0×10^3 3.0×10^3 5.0×10^3 3.0×10^3	92,444 333,394 324 7,236 123,160 3,953 122,240 14,500 34,600 8,120 42,210		(45) (46)	Calculations based on assay data per square foot and surface area of components assayed.
Total vehicle less forebody and payloads	1.2×10^6		2.0×10^6	811,846			
[illegible] [illegible] Propulsion Organic [illegible] Organic [illegible] payload Structure [illegible] Stabilization [illegible] Office Support information [illegible] Inorganic payload Science Communication Support and data Other				$164,729 \times 10^3$ $120,358 \times 10^3$ $12,117 \times 10^3$ $93,243 \times 10^3$ $88,456 \times 10^3$ $1,061 \times 10^3$ 663×10^3 $5,823 \times 10^3$ 36×10^3 $1,939 \times 10^3$ 325×10^3 $5,833 \times 10^3$ 726×10^3 $2,656 \times 10^3$ $1,471 \times 10^3$ $1,137 \times 10^3$		(47)	Calculations based on estimates of cleanroom fallout rates and assuming surface decontamination by ETO
[illegible] (Assembly [illegible]) AIMP prototype Spacecraft Controlled section AIMP flight spacecraft during assembly AIMP flight spacecraft	Controlled section		Class 100 = none/6.0×10^2 Class 100 Before decontamination After decontamination	1,794 35 7,077 416	186 33	(48) (51) (51) (51)	Assay Assay Assay Assay
Total microbial burden of the AIMP flight spacecraft after decontamination					268,336	(51)	

*All units in number of organisms (low/high) per square feet, unless otherwise indicated
**Pilot spacecraft was a mock-up consisting of typical spacecraft hardware and components.
***The facility was operating at a Class 10,000 level during this program.

Table 3-5. Bioburden Catalog, Part III-Supplementary Data: Fallout onto Test Surfaces

Manufacturing Environment / Experimental Surface	Normal Manufacture	Controlled Environment Facilities: Class 250,000	Class 100,000	Class 100	"Bioclean" Facility	Reference	Comments
Stainless Steel Strips							
Cross Flow Room (2)	*			300/22 700 (1)		51	(Assay)
Down Flow Room (2)				0°/1,512		51	(Assay)
Plotting Room (2)	3.000/40,000					51	(Assay)
General Electric Facilities	$4.0 \times 10^3/4.0 \times 10^5$		$1.0 \times 15/6.9 \times 10^1$	$0.33 \times 10^0/0.9 \times 10^0$	None/2.25×10^0	45	(Assay) class 100,000 = Class 10,000 here
Cutler Industries			$3.0 \times 10^1/7.0 \times 10^1$	$0.6 \times 10^0/11.6 \times 10^0$		45	(Assay)
Sandia Lommar Down Flow				None/2×10^0		45	(Assay)
During Assembly (1 in. x 2 in. stainless steel strips)			0/297			52	(Assay) U.S. Public Health Service studies
Handled by Personnel (1 in. x 2 in. stainless steel strip)			0/241 (Class 10,000)			52	(Assay) Class 100,000 Class 10,000 hours (Same individual different s.s. strip)
University of Minnesota Laboratory (1 in. x 2 in. stainless steel strip)	655/2400 (3)					52	(Assay)
Martin Co. Middle River, Md	19,000 (4) 19,000 (5)		600 (4) 810 (5)			54	(Assay)

1. All units in number of organisms (low/high) per sq. ft. unless other wise indicated
2. Testing consisted of a 10 day time period including weekends. Values depend upon number of people, material, location of strips, in the rooms
3. Total mesa count for strips plated immediately after exposure, time from 1 to 14 days. Average based on five (5) 1 x 2 in. s.s. strips
4. Five week cumulative exposure period during 47th to 52 week of the study
5. Fifty-two week exposure

SECTION 4

EFFECTS OF INTERPLANETARY ENVIRONMENTAL FACTORS ON THE VIABILITY OF MICROORGANISMS

4.1 BACKGROUND

The flight through space has several conditions which can effect the viability of microorganisms: vacuum, desiccation, radiation, temperature, and long time periods. For accurate determination of their combinatorial effects, the conditions of outer space should be simulated, as a whole, or actually used for studies. Unfortunately, this has not been done, and the kill estimations must be based upon studies with, at most, only two factors considered concourrently.

4.2 VACUUM EFFECTS

Generally, vacuum, whether applied to frozen suspensions of microorganisms, i.e., freeze-drying, or as desiccation at room temperature, is a good means of preserving microorganisms (Reference 14). Preservation can be effective for periods of from several months to many years. There is no danger of growth and reproduction because of the dry, nonnutritive conditions. If this were the only condition which would exist during space flight, similar effects could be postulated. The die-off rate of spores or organisms under vacuum at low temperatures has not been quantitatively established although it is known that organisms under such conditions do die off. Effects of high vacuum are discussed in Section 4.3.5.

4.3 EFFECT OF TEMPERATURE ON THE VIABILITY OF MICROORGANISMS

Because temperatures below freezing tend to preserve viable microorganisms and temperatures above 0°C tend to reduce viable microbial populations, a spacecraft will undergo some temperatures which could affect the number of microorganisms remaining viable on or in the spacecraft; therefore, it seems logical to evaluate the potential of such an effect on the reduction of spacecraft bioload.

The temperature ranges expected to be present on and in the Voyager spacecraft should not cause extensive kill of viable microorganisms, particularly spores. Entrance through a

planetary atmosphere can generate extremely high surface temperatures on the entering body. A separate analysis of the effect of those temperatures upon free particles (spores or spores attached to particulates) is being performed elsewhere in the Planetary Quarantine Study at General Electric.

Another phase of the flight in which temperature could play an important role regarding the potential killing effect on viable microbial spores is the effect of rocket motor temperatures on any contaminant in the motor or propellant. That aspect of the current Planetary Quarantine Study program, covering some practical tests being made to determine such effects, will be reported separately.

4.3.1 LOW TEMPERATURE

The survival of many microorganisms after exposure to temperatures near absolute zero has been documented (Becquerel, 1950, Reference 57, and Heckly, Reference 58).

4.3.2 INTERMEDIATE TEMPERATURES

A compilation of known data has been made over the temperature range thus far investigated (Figure 4-1). Some of the data inputs to Figure 4-1 are taken from experimental results, others are point data, and portions were extrapolated. However, it represents the best information, even with the acknowledged reservations, (Wang et, al., 1964, Reference 55; Schalkowsky, 1966, Reference 56), that are available and allows some basis for interpretation of postulated effects.

The reservations of most concern specifically are:

a. Extrapolation of death-rate data over a wide temperature range with the use of thermal-death-time curves can lead to serious errors.

b. At long heating times, a log-normal distribution model of the survival times of organisms in heat sterilization (in which the probability of inactivation, as a function of exposure time, is log normally distributed) appears more accurate for extrapolating to low survival probabilities than the usual logarithmic survivor curves and is generally more conservative.

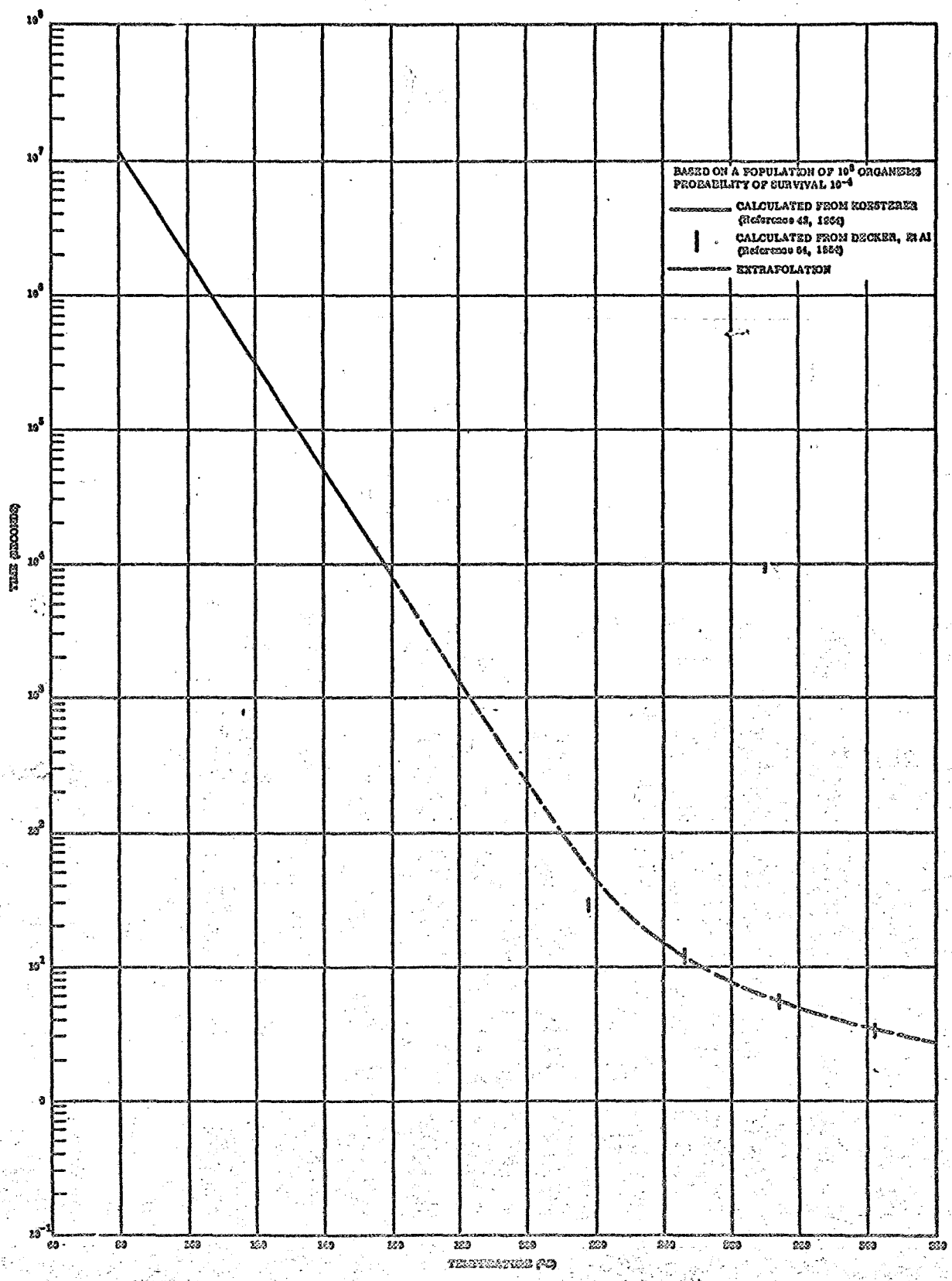

Figure 4-1. Thermal Death Times for Populations of Dry Spores of *Bacillus subtilis* var. *niger*

4.3.3 ALTERNATE FREEZE - THAW TREATMENT

Several authors (Young et. al., Reference 44, 1963; Hawrylewicz et al, Reference 61, 1965; Packer et al, Reference 37, 1963) have reported the ability of various microorganisms to withstand freeze-thaw cycles for long time periods. Some selection of the organisms tested was observed and the conditions in some cases, depending on other factors simulated, allowed the organisms to grow and reproduce.

4.3.4 ULTRAHIGH TEMPERATURE

A basis for extending the curve in Figure 4-1 to higher temperatures is uncertain. Data from other studies such as Melpar study (Reference 60) on the shock of microorganisms and some Fort Detrick studies (Reference 62) on the ability of microorganisms to withstand exposure to high temperatures are available and allow some basis for judgment.

The Melpar experiments in which spores of Bacillus subtilis var. niger, were exposed to extremely high-temperature gas, indicate that some microorganisms can withstand 1060°C for 8.1 milliseconds, 1510°C for 2 milliseconds, 1220°C for 1.96 milliseconds, and 2035°C for 1.84 milliseconds. The Melpar study, however, did not determine the length of time necessary for sterilization at these temperatures nor was it intended to quantitate survival, and one may only assume that the time for sterilization lies above 1 to 2 milliseconds. It is believed that at some temperature, the heat transfer properties of microorganisms become the limiting factor for sterilization in such short time, but neither the time nor temperature have yet been found.

Several factors influence the effects of heat on microorganisms. The material which the bacterial spores are on, or in, can change the time required for sterilization by as much as a factor of 3 (Reference 13). Moisture increases the heat sensitivity of microorganisms and a hot flowing gas is more efficient than stationary hot gas (Reference 42).

A better way of considering thermal effects may be in terms of inactivation energy. Plotting the log of D (the time to kill 90 percent of a population) against 1/T has shown that the

Arhenius rate expression is applicable for all organisms and substrates. (Reference 2) Thus, the inactivation energy for dry heat, E_{dh}, may be determined by

$$\frac{1}{D} = Ae^{\frac{-E_{dh}}{RT}}$$

where

R = gas constant

A = constant referred to as frequency factor

T = temperature (^{o}K)

Having determined E_{dh} for any one temperature, the combinations of time (t), and temperature "which will give the desired probability of contamination for an initial population of microorganisms with a certain resistance parameter" (Reference 2) may be determined by

$$n = te^{\frac{-E_{dh}}{RT}}$$

where n = kill requirement. The necessary energy for bacterial spore inactivation appears to be in the range of from 35,000 to 40,000 calories/gram-mole (Reference 63).

4.3.5 ULTRAHIGH VACUUM AND TEMPERATURE EFFECTS

Many studies have been made on the combination of these effects either as a primary objective or because when investigating the effects of ultrahigh vacuum, one cannot divorce the temperature factor (Morelli, Reference 34, 1963; Porter et al, Reference 40, 1961; Silverman et. al, Reference 20, 1963). Gloger et al (Reference 35, 1964) and Packer et al (Reference 37, 1963) all report the survival of both vegetative and spore forms of one or several microorganisms for as long as several months at temperatures ranging up to room temperature (25°C) and vacuums as high as 10^{-10} torr and for shorter times (4 to 8 days) at temperatures up to 62°C and 80°C. All of the points (temperature-time) above which Silverman (Reference 20, 1963) found no organisms surviving fall below the curve in Figure 4-1. In addition, where the data for samples allows the comparison of

organisms exposed to ultrahigh vacuum and under ambient pressures, it appears that the time to sterilize is less under vacuum. Not enough information is available at this time to estimate die-off rates under ultrahigh vacuum conditions.

The substrate on which organisms are exposed will have effects upon their survival and the extremes which they can endure (Reference 20).

4.4 RADIATION EFFECTS ON THE VIABILITY OF MICROORGANISMS

4.4.1 BACKGROUND

The outer surfaces of interplanetary spacecrafts will be exposed to solar ultraviolet and soft X-rays in those positions in which they face the sun. (References 5, 28, 30, 31 and 43). It is suspected that many of these exposures may be bactericidal or sporicidal (References 2, 3, 4, 8, 10, 12, 13, 14).

4.4.2 LETHAL RADIATIONS IN SPACE

The several types of radiation in space which can be lethal include: a) cosmic radiation, which primarily consists of protons, alpha particles, and some heavier particles and b) electromagnetic radiation, which is lethal to microorganisms in the ultraviolet, X-ray, and gamma ray ranges.

Galactic cosmic radiation has a stable flux, is of high energy, and is omnidirectional. Solar cosmic radiation, however, is erratic (nine major solar flares in the last 20 years and about eight minor flares per day), of relatively low energy, and directional. Solar electromagnetic radiation is stable and directional (Reference 1).

The solar ultraviolet radiation flux at the surface of Mars is a vital factor of the ecology. There is little information available on the effects of the flux (Reference 43). Evans (Reference 30, 1966) reports that no gases absorbing in the ultraviolet down to wavelengths of 2400 Å have been detected in the Martian atmosphere. The surface of the planet is irradiated, during some fraction of the Martian year with essentially unfiltered sunlight.

4.4.3 BIOLOGICAL EFFECTS OF RADIATION

The effects of radiation depends upon the number of organisms irradiated and on the dosage level. The dose actually absorbed by organisms, and not the incident dose, determines the fraction killed. Thus, the chemical receptor for the radiation must have the same absorption spectrum as the photochemical event. For ultraviolet radiation, the wavelength primarily absorbed by the pyrimidine and purine bases of nucleic acids, and the tyrosine and tryptophane amino acids in proteins, has the greatest inactivating effect (References 2 and 14). This wavelength, in most cases, is a 2650 Å, with the 2537 Å wavelength radiation being about 85 percent as effective (2537 Å wavelength radiation has been employed almost solely in experimentation because low-pressure mercury lamps, which are commercially available, produce this wavelength). (References 4 and 14.)

The lethality of high-energy radiations is due to ejection of electrons from atoms, producing ion pairs, which in turn leads to numerous, apparently unrelated chemical changes. Some of these changes (inactivation of enzymes, interference with enzyme and/or nucleic acid synthesis, production of extracellular or intracellular poisons, and lethal mutation) are effective in inactivating the microorganism. The chemical change which is actually responsible for inactivation at any given time is dependent upon the environment.

A reversal of radiation injury (photoreactivation) can result from the subsequent or simultaneous exposure of the organisms to visible (Reference 2) or near ultraviolet light (References 3). Photoreactivation after ultraviolet radiation exposure is never 100 percent and the reactivated fraction depends upon the ultraviolet/visible dose received. No systematic work has been done employing continuous doses of ultraviolet light to determine if the photoreactivation phenomena can be overcome. If visible light is incident upon a population of microorganisms at the same time as the ultraviolet radiation, the inactivation rate per unit dose of ultraviolet radiation is reduced by some constant factor. Photoreactivation has been observed on a molecular as well as an organismal level, and the effect seems to be specific for microorganisms. Photoreactivation is temperature-dependent, increasing as temperature decreases, and the mechanism is unknown.

One important aspect to consider in this discussion is that the potential effects evaluated in this report are for microorganisms in a nonnutritive environment. Siegel (Reference 9) has pointed out that various species of terrestrial microorganisms (bacteria and fungi) have grown after exposures up to seven equivalent martian days (1.0 EMC = 4.3×10^8 erg-cm^{-2}). It is expected that organisms, supplied with an adequate source of nutritive substrates, could repair and overcome such exposures. The doses of X-rays, gamma rays, and ultraviolet radiation required to kill various microorganisms are summarized in Table 4-1. A 4.4×10^6 ergs/cm^2 dose of ultraviolet radiation was sufficient to kill in all the microorganisms tested. (Reference 8). A mean lethal dose for Micrococcus radiodurans, an exceptionally radio-resistant bacterium, has been reported by Setlow and Duggan (Reference 39, 1964) to be 6000 ergs per square millimeter (at 2652 Å). Horowitz et al (Reference 28, 1967) report that doubling "this dose would reduce the survival to less than 0.1 percent and that doses in this range would be accumulated in a matter of minutes under direct Martian sunlight." Table 4-2 displays the dosage versus kill relation for less than total annihilation of a population.

To estimate the effect of ultraviolet radiation on the viability of a population of microorganisms during space flight, a D-value (dose to reduce population by 90 percent) for the population must be available. Because no D-value has been determined for a representative population, which might be present on a spacecraft, the highest D value, for other than M. radiodurans, from the literature has been employed (Reference 4). Table 4-3 expresses ultraviolet dosage effects in terms of these D-values. From this table, Figure 4-2 has been plotted to permit the determination of the effects of exposure to ultraviolet radiation.

Table 4-1. Doses of Various Radiations on Various Microorganisms

(a)
ULTRAVIOLET: Erg/cm^2

	Steri-Tronics 100% Kill (Reference 12)	Buttolph 99% Kill (Reference 4)	Hollaender 90% Kill (Reference 13)	Phillips & Hanel 100% (Reference 13)	Setlow Duggan MLD* (Reference 39)
Mold - Vegetative		162000-1300000			
Spore	110000-3300000				
Bacteria - Vegetative					
Spore	57200- 264000	16200	11300-197000	98400	6000000 for *M. radiodurans*
Virus	34000-4400000		27300-120000	480000	
Yeast	66000- 176000				

*MLD = Mean Lethal Dose.

(b)
X-RAY: rad to Kill 100%

	Dunn, et al (Reference 15)
Bacteria - Vegetative	2.26×10^4-4.65×10^5
Spore	4.65×10^5-1.86×10^6
Mold	2.33×10^5-9.3×10^5
Yeast	2.33×10^5-1.4×10^6
Number Exposed	10^6 - 10^8

(c)
GAMMA RAY: rad to Kill 100%

	Lawrence, et. al. (Reference 16)	Goldblith, et. al. (Reference 17)	Tarpley, et al (Reference 18)
Bacteria - Vegetative	2.69×10^5	9.13×10^4	
Spore	1.9×10^6	7.3×10^5	2.54×10^3
Mold	3.16×10^5		
Yeast	3.16×10^5 **		
Number Exposed	10^7 - 10^9	10^8	10^9

**99.99999% Kill

Table 4-2. Doses of Ultraviolet Radiation Required to Kill Fractions of Various Types of Microorganisms.

(Reference 4)

Killed Fraction (Number Dead/ Initial Number)	Dose (in microwatt-sec/cm^2 or cm^3)		
	Resistant Fungi	Dry Bacillus Coli and Viruses	Some Fungi, Bacillus Coli In H_2O And B. Subtilis
0.9999	192000	3000	24000
0.99	96000	1500	12000
0.90	48000	750	6000
0.70	24000	375	
0.632		300	

Table 4-3. Lethal Doses (in D values) of Ultraviolet Radiation for Various Types of Microorganisms

Survival Fraction (1-Killed Fraction)	Dose as a Multiple of D (Dose Required to Kill 90%)		
	Resistant Fungi	Dry B. Coli and Viruses	Some Fungi, B. Coli In H_2O B. Subtilis
10^{-4}	4D	4D	4D
10^{-2}	2D	2D	2D
10^{-1}	D (48000)	D (750)	D (6000)
$10^{-.523}$	0.5D	0.5D	
$10^{-.448}$		0.4D	

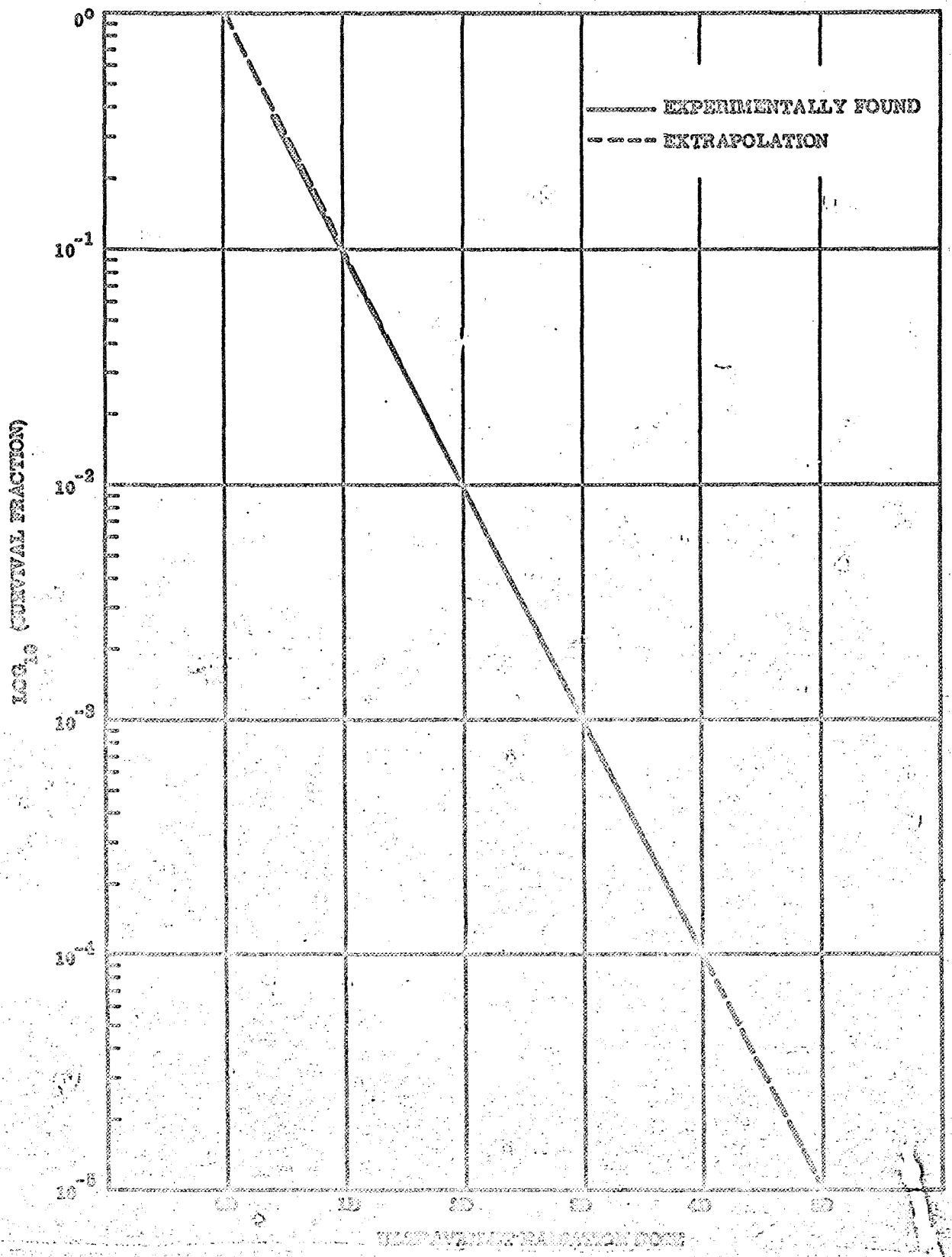

Figure 4-4. Survival Curve of Microorganisms to Ultraviolet Radiation

The equation for the exponential curve in Figure 4-2 may be derived from Table 4-3 as follows:

$$x = nD$$

$$y = 10^{-m}$$

$$m = n$$

Where: x = the ultraviolet dose received

y = the survival fraction

n = real number

m = a real number

D = the dose required to kill 90 percent of a population

Then, $n = x/D$

$m = x/D$

and, $y = 10^{-x/D}$, the equation relating survival of microorganisms to the dose of ultraviolet radiation. Although the data reviewed indicates that death rates do follow exponential curves, deviations are acknowledged to exist. Two factors are believed to effect the curve in Figure 4-2 at high survival levels. There is probably a "minimum lethal dose," or a dose below which no organisms die from radiation effects. The minimum lethal dose would deflect the actual curve to the right of the projected formula curve as the survival fraction approaches 1.0.

Natural die-off (here considered to be death caused by any factor other than ultraviolet radiation) has little effect on the curve when large percentages of a population are killed; but when only a small fraction is killed by ultraviolet radiation, natural die-off becomes significant. As the survival fraction approaches 1.0, natural die-off deflects the actual curve to the left of the formula curve.

Figure 4-2 shows the natural die-off deflection occurring somewhere between 10 percent and 30 percent survival. There is probably little or no minimum lethal dose deflection in the solid line part of the curve; but because of these antagonistic effects and lack of information, any extrapolation of the line to higher than 36.8 percent survival (less than 63.2 percent kill in Table 4-2) would be highly speculative.

4.4.4 SAMPLE CALCULATION OF RADIATION DOSE IN SPACE

A simple calculation, showing the effects of ultraviolet radiation in space may be performed as follows:

a. Intensity of 2537 Å wavelength radiation in space (Reference 5) = 9.064×10^3 microwatts/cm^2.

b. Typical time for flight from Earth to Mars is 180 days or 1.552×10^7 seconds.

c. Expected total dose, from equations (a) and (b) = 1.41×10^{11} microwatt-seconds/cm^2.

d. The dose necessary to produce a survivor probability of less than 10^{-4}, for example, given an initial population of 10^8 microorganisms and employing:

1. The equation ($y = 10^{-x/D}$) can be evaluated by:

$$\frac{10^{-5}}{10^8} = 10^{-x}/4.8 \times 10^4 \text{ microwatt-seconds/cm}^2$$

and solving for x; $x = 6.24 \times 10^5$ microwatt-second/cm^2

Given the above expected dose in space. The survival fraction (y) to be expected

$$\text{is } y = 10^{\frac{-1.41 \times 10^{11} \text{ microwatt-seconds/cm}^2}{4.8 \times 10^4 \text{ microwatt-seconds/cm}^2}}$$

$$= 10^{-2.94 \times 10^6}$$

2. The use of Kelly's (Reference 4) modification of Schmidt's equation for calculating a sterilizing dose (the method conventionally used to estimate such parameters) (F) is:

$$F = D_T (\text{Log } N_o + 1 + \log 1/P$$

where D_o is the decimal reduction dose at temperature T_o (48,000 microwatts-seconds/cm^2) (Reference 4), (the highest value for 2537 Å noted in the literature) and P is the probability of a single surviving organism in an original population of N_o

$$F = 48,000 (\log_{10} 8 + 1 + \log 1/10^{-4})$$

$$F = 6.24 \times 10^5 \text{ microwatt-seconds/cm}^2$$

Solving for P, using the expected dose of 1.41×10^{11} gives a probability of survival of approximately $10^{-2.24 \times 10^6}$

The above calculation presumes exposure of the microorganism population to the ultraviolet radiation for the full duration of the mission. The time for a dose of 9.0×10^3 microwatts/cm^2 (of 2537 Å) to sterilize a population of 10^8 organisms with a $F = 6.24 \times 10^5$ microwatt-seconds/cm^2 would be 1 to 2 minutes.

The effects of intermittent radiation should be additive in effect if the possibility of photoreactivation is neglected. Furthermore the calculation does not consider the significant possibility of the microorganisms being shielded from the radiation. Indications are that a shield of a material such as aluminum of 700 Å thickness or greater would be totally protective (Reference 8). The potential synergistic effects of ultrahigh vacuum and radiation, (Reference 27) if known, would probably be another ameliorating factor.

In a similar calculation, Horowitz et al (1967 Reference 29) indicate that based on Allen's (1966, Reference 30) report of an integrated solar flux in the lethal spectral range of 2400 to 2900 angstroms exceeding 20 ergs per square millimeter per second at Mars, an exposure period on the order of minutes would be adequate to kill a population of M. radiodurans.

The curve in Figure 4-2, since the D value parameter is time dependent, will allow for the determination of UV effects for shorter time interval exposures.

X-rays and gamma rays in doses of 1.86×10^6 rad have been shown to kill six logs of highly resistant populations of 10^{10} organisms, or less (Reference 8).

A dose of about 2.54×10^6 rad will probably destroy all organisms. Assuming an exponential death rate, a dose of 1×10^7 rad of ionizing radiation will reduce the original microbial count by a factor of 10^{-13}.

The percentage of the microbial population of a spacecraft other than on the surface, which is actually killed by radiation depends upon the ability of the high-energy electromagnetic and cosmic radiations to penetrate the structure of the craft. The Russians (Reference 9), by exposing lysogenic bacteria on their Vostok flights, have shown that interior portions of a spacecraft are subject to irradiation. Thus, there should be a gradient of radiation kill from the outside to the inside of the bus.

4.4.5 OTHER FACTORS:

4.4.5.1 Vibration

Phage induction on the Vostok flights was greater than what the actual dose would be expected to cause; laboratory studies (Reference 10) were conducted to examine the effects of radiation accompanied by vibration. The same techniques (observation of phage induction) show that vibration alone has no effect on the lysogenic bacteria, but while in combination with or before irradiation, vibration increases bacteria sensitivity to radiation. Weightlessness may have a similar effect, but has not been studied to date.

4.4.5.2 Radiation and Heat

The effects of gamma radiation and heat have been shown to be better than additive with regard to the killing of microbial spores (References 8 and 11). Since radiation doses can be absorbed in the cold regions of the spacecraft as well as the warm, the assumption of an additive kill due to heat and radiation is certainly a conservative estimate as to what might happen in space. The spacecraft effects of vibration and radiation would appear to make radiation doses, cited earlier, conservative figures for total kill.

4.4.5.3 Radiation and Ultrahigh Vacuum

The effect of ultrahigh vacuum to remove water and volatile external spore constituents, as well as internal components that can pass the spore coat barrier do not, in Silverman's evaluation (Reference 20), account for the reduction in radioresistivity (to gamma rays) of bacterial spores. If such an effect is evidenced by exposure to the vacuum of space in combination with the radiation exposure, it would allow that the above estimates of the effects (or calculations) would be on the conservative side.

4.5 SIMULATION OF EXTRATERRESTRIAL ECOLOGIES

There is very little information available in this area at the present; and the reports which are available (References 7, 19, 20, and 37) have attempted to simulate only some of the Martian ecology, at least the parameters which can be estimated. The general conclusions are that various microorganisms have survived freeze-thaw cycles, atmospheres of high inert gas concentration and low moisture content and pressures less than a fraction of an atmosphere as well as total ultraviolet dose of 10^9 erg cm^{-2} (References 31, 32, 33 and 44).

Ultraviolet radiation (with wavelengths in the range of 2650 - 2800 Å) have been shown to sterilize aerosols of microorganisms and exposed smooth surfaces in short exposures (References 2, 3, 4, 12, 13 and 14). In fact, Olson et al (Reference 38) could not recover viable spores B. subtilis var. niger ejected from contaminated surfaces by simulated micrometeorite impaction with subsequent exposure to ultraviolet radiation, whereas there was survival of "natural" co[illegible] from handling and spores of B. subtilis var. niger on solar panels and aluminum discs. The most plausible explanation for organisms surviving on the materials was that some form of mechanical shielding was protecting them.

SECTION 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The conclusions of this study are:

a. Sparse information is available concerning the microbial population on spacecrafts or spacecraft hardware. However, the catalog presents bioassay data and estimates of the bioburden of spacecraft hardware manufactured under varying degrees of contamination control. From these data, an estimate can be made for a high and low level of potential spacecraft contamination. A relative number or range of numbers of organisms can be assigned with caution to the various parts and to the total spacecraft. It should be noted that the level of cleanliness employed, as tabulated in the Bioburden Catalog (Tables 3-3, 3-4, and 3-5), is itself somewhat undefined; i.e., clean rooms may be "rated" at one level and "operated" at some other level. Consequently, no exact correlation between "clean room rating" and microbial bioload can be made from the data.

b. Of the extraterrestrial conditions that have a potential effect upon the viability of microorganisms, only two are significant, namely, radiation applied to free unshielded organisms and elevated temperatures attained only under certain specific phases of the flight program.

5.2 RECOMMENDATIONS

On the basis of the surveys and studies reported herein, it is obvious that additional data are needed on most aspects before a firm decision is to be made concerning the effect of these factors on the viability of microorganisms in extraterrestrial environments.

Specifically, the most significant areas requiring further investigation are:

a. Determination of die-off rate of microbial spores under simulated extraterrestrial conditions as a result of vacuum, dessication and anticipated radiation levels.

b. Verification of the thermal death times of microbial spores exposed to ultrahigh temperatures for very short times.

c. Determination of whether photoreactivation phenomena are actually involved or can be overcome by long term exposure to ultraviolet radiation under nonnutritive conditions.

SECTION 6

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