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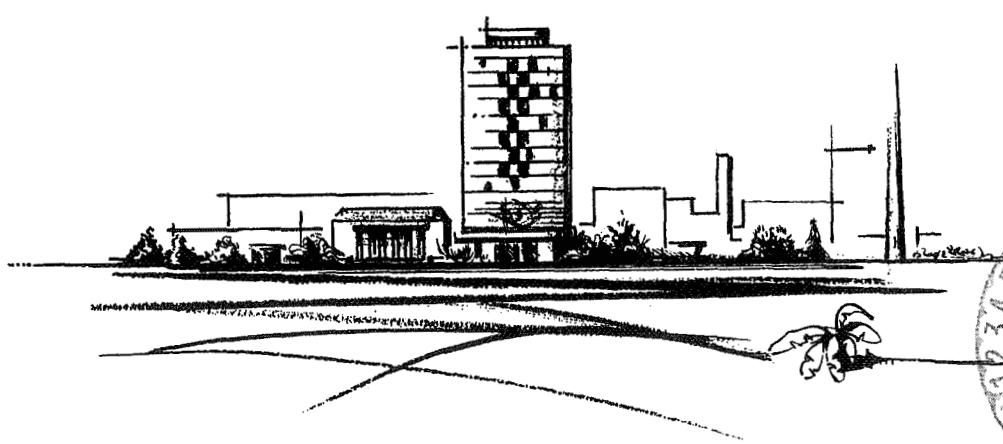
RESEARCH REPORT

INVESTIGATION OF SPACECRAFT MATERIALS
THAT SUPPORT MICROORGANISM GROWTH

(Contract No. NAS8-30504)

to

GEORGE C. MARSHALL SPACE FLIGHT CENTER
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MARSHALL SPACE FLIGHT CENTER



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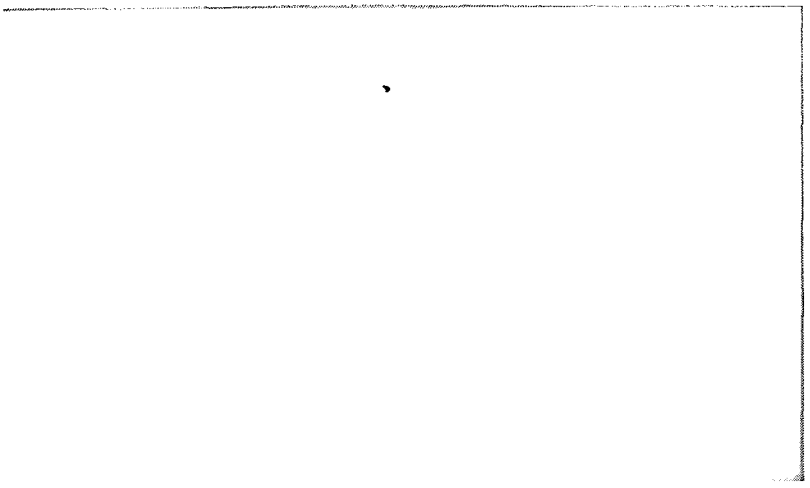

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SUMMARY REPORT

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June 17, 1970

H. T. Kemp and C. W. Cooper

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FOREWORD

This report was prepared by Battelle Memorial Institute, Columbus Laboratories under Contract No. NAS8-30504, entitled "Investigation of Spacecraft Materials That Support Microorganism Growth" for the George C. Marshall Space Flight Center of the National Aeronautics and Space Administration. The work was administered under the technical direction of Mr. Frederick J. Beyerle, Program Monitor. The research described in this report was conducted during the period from September 1, 1968, to June 30, 1970.

ABSTRACT

Seventeen coatings were selected as being representative of 166 such materials applied externally on spacecraft. The coatings were applied on aluminum rods and evaluated in the laboratory to determine whether they were resistant to microbial attack and whether nutrients from the coatings were available for the growth of microorganisms. All of the coatings contained nutrients suitable for microbial growth or allowed survival of two or more species studied. Some of the coatings, e.g., fungicide-containing varnishes, a phenolic butyrate, and a polyimide, appeared to be somewhat resistant to microbial attack; while epoxy, acrylic, silicone, silicate, and polyurethanes respectively, in decreasing order of resistance, appear to be more susceptible to attack. Microbiocide incorporation into these coatings was recommended for improved inhibition of microorganisms in regard to spacecraft sterilization and for increased protection from deterioration by microorganisms.

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INTRODUCTION

The presence of microorganisms on external coated surfaces of unmanned spacecraft is undesirable for two reasons:

(1) The microorganisms can survive the adverse conditions of interplanetary spaceflight and contaminate other planets. This is considered undesirable since the contaminants could be a serious threat to indigenous planetary life forms, if present.

(2) Prior to launch, during prolonged storage periods, and following impact or landing on a planetary surface, the microorganisms could severely degrade spacecraft materials that are not inherently resistant to microbial attack and significantly alter coating performance. Microbial growth and deterioration of materials presumes conditions favorable to microorganisms, such as temperature, humidity, and other environmental conditions.

Coatings are applied to external surfaces of spacecraft for varied reasons, including passive thermal control, radiation protection, radar reflectance, and to protect underlying metal substrates from chemical attack, oxidation, and corrosion. These coatings are selected for particular spacecraft on the basis of laboratory, and sometimes flight, data in which some indication of their ability to withstand and perform in the space environment is obtained. In addition to special requirements for each application, such as optical or electrical properties, these coatings must have excellent adhesion, stability to heat and radiation fluxes, and resistance to erosion from rain droplets or atmospheric particles.

Coating materials for spacecraft can be categorized as follows: (1) metals, (2) inorganic compounds, and (3) organic and semiorganic compounds. Those coatings which are further described as paints were the subject of this research program. By definition, various types of paints may fit into each of the above categories. A listing of individual paint coatings used on spacecraft is long indeed, considering past vehicles or ones now being used or intended for use:

Orbiting	Deep Space and Lunar Orbiting	Manned
ATS I-III	IMP	Apollo
Discoverer	Lunar Orbiters I & III	Gemini
Echo	Mariner I-IV	Mercury
Explorer I-XVII	OSO I-III	MOL
Geos	Pioneer	MORL
Nimbus	Ranger-Mariner	Sky Lab
OAO	Surveyor	
OSO I-III	Voyager	
OGO		
Pegasus I-III		
POGO		
Samos		
Tiros		
Transit		
Vanguard		

In this study, those paint coatings used only on unmanned, orbiting, and deep space vehicles were considered.

A "typical" interplanetary spacecraft might have, depending on its mission, the following coated components:

Antenna - low α_s/E paint

Experimental sensors - low α_s/E paint

Exposed electronic components - black paint

Thermally isolated booms - black paint with aluminum foil

Sun sensors - vacuum-deposited aluminum

Isolation covers - aluminum mylar multilayer

Isolation band - aluminum mylar or polyimide multilayer

Equipment platform - aluminum honeycomb with high emittance bottom

Etc.

Only externally applied paint coatings were evaluated in this study.

All organic materials can be susceptible to attack by microorganisms. Many inorganic materials such as metal alloys contain trace amounts of metal salts which when leached out can provide essential mineral elements that may stimulate microbial growth. Thus, a combination of organic and inorganic materials, such as those found on external surfaces of spacecraft, can provide ample nutrients and essential elements for microbial growth. Furthermore, particulate nutrients in the form of dust, pollen, microbial spores, etc., can settle on these surfaces and also provide nutrients to support growth. Diurnal meteorological and other fluctuations of temperature and humidity can cause water condensation on spacecraft surfaces prior to launch. Pooling of the condensate water would tend to concentrate water-soluble nutrients in localized spots on the spacecraft. If the spacecraft is exposed before launching to the open atmosphere, rainfall and dew would tend to accelerate this process.

It is important to note that seldom is a single factor responsible for failure of this type of material. Invariably, the combined effects of many adverse factors must be taken into account.

Microbial contamination of planets and other cosmic bodies is of concern to the U. S. space exploration program because these contaminants may be pathogenic to or may have adverse effects on extraterrestrial life forms, as well as providing a potential hazard to man in the form of mutants of the contaminants. Additionally, contaminants could well result in false-positive indications of the presence of extraterrestrial life in early stages of planetary exploration. These considerations are true whether a contaminant is borne externally or internally, since the possibility of a space vehicle wreck by impaction on planetary surfaces is not unexpected and, in fact, sometimes planned. The microorganisms on externally-applied paints probably constitute a small proportion of the total microbial load of spacecraft.

A considerable technology has developed from attempts to minimize or eliminate microorganisms from planetary and deep space probes, especially those that may impact. Dry heat, autoclaving, exposure to ethylene oxide, and to mixtures of gases such as methyl bromide/ethylene oxide, or combinations of these treatments for component parts, have been used in "sterilizing" space vehicles. Assembly of spacecraft in ultra-clean, nearly sterile rooms is another technique used to minimize microbial and other types of contamination.

Microbial contaminants within a space vehicle can result in deterioration of most organic materials and can become an undesirable particulate load with respect to electronic and mechanical equipment and similarly to man in later manned space probes. The research reported here on the microbiology of external spacecraft paint coatings could be extended and applied to all materials used in spacecraft.

A further technique for minimizing microbial populations aboard spacecraft is the use of materials that would not support the growth of microorganisms and that might even be inhibitory to them. This is one of the long-range aims of this program.

Although ambient temperature, humidity, and gas composition of the atmosphere within a space vehicle are controlled within established required limits, these variables may fluctuate widely in niches throughout the vehicle, and to some extent on external painted surfaces. This may be due to the inaccessibility or remoteness of some areas to environmental control mechanisms and to brief moments of aerodynamic heating. Fluctuations in temperature and humidity can result in condensation and pooling or accumulation of water. Thus, a microclimate suitable for the growth of microorganisms can become established with a resultant microbial load of undesirable proportions. This sequence could be controlled to some extent by engineering design, i.e., minimizing the niches and microenvironmental fluctuations. However, minimizing nutrients in paint formulations or use of paints with microbiocidal properties is a more convenient and more promising approach. A wide range of effective agents is available for imparting microbiocidal properties. Three of the many criteria that would be used in selecting these microbiocides are very low volatility or low-vapor pressure, lack of corrosiveness, and very low human toxicity. Microbiocide incorporation into selected polymers is a logical follow-on program to the research reported here.

OBJECTIVES

The primary objectives of this program were to:

(1) Survey comprehensively the literature on paint coatings now used externally on spacecraft surfaces and those experimental coatings that are leading candidates for this application; summarize the available physical and chemical data on these coatings; and summarize the available information on these coatings for their ability to support growth or to inhibit the growth of microorganisms.

(2) Evaluate selected spacecraft paint coatings in laboratory studies for their growth-supporting or biocidal properties using selected, representative strains of bacteria and fungi.

In agreement with NASA, objectives concerned with chemical identification of coating extractables and recommendations for formulation changes were omitted from this program. This was primarily because coating formulations are proprietary and are closely held by the individual suppliers.

PROGRAM DESCRIPTION

The two phases of the program were emphasized as required in consultation with and with the approval of the NASA technical monitor. Minimal information was available from the scientific literature on the nutrients or biocides in spacecraft coating materials. As a result, less effort was required in Phase I (literature survey) and we proceeded almost immediately to Phase II (laboratory evaluation). Subsequent phases will depend upon further NASA funding.

PHASE I. LITERATURE SURVEY

Paint coatings utilized on external surfaces of spacecraft are enumerated in a wide variety of sources including supplier's data sheets. Substitution or trade-offs for various reasons, including flammability and radiation resistance, are continually made to meet revised specifications or to improve material performance in the space environment. Data concerning the paint coatings currently used in space vehicles were summarized in a standardized format, as shown in Figure 1. Precise identification of each material is important since, for example, the slightest modification of a coating formulation or metal alloy might result in a drastically changed rate of nutrient, metal salt, or microbiocide release.

Computer searches based on formal work descriptors (standard vocabulary) as well as an informal description of the subject matter were requested from Defense Documentation Center, Alexandria, Virginia, and from the Machine Search Branch, NASA, Scientific and Technical Facility, College Park, Maryland. Descriptions and compositions of spacecraft materials and techniques for their study in the proceedings of the Society of Aerospace Material and Process Engineers (SAMPE) were studied for applicability to this program. Information was also sought informally from the Defense Metals Information Center, Defense Ceramics Information Center, and the Radiation Effects Information Center located at Battelle-Columbus. Additionally, scientists and engineers at Battelle-Columbus engaged in aerospace research were queried for pertinent information and advice.

Sources of microbiological information on this subject on hand included bibliographies from Plastek at Picatinny Arsenal; the now defunct Prevention of Deterioration Center; the American Chemical Society, Division of Rubber; the U. S. Naval Gun Factory; and the National Bureau of Standards. The other more usual abstracting sources were also consulted.

Coatings:

Trade name:

Source:

Chemical nature of formula:

Substrate:

Thickness, mils:

Solar absorptance at 530° R (α_s):

Hemispherical emittance at 530° R (E_H):

Absorptance - emittance ratio (α_s/E_H):

Thermal stability:

Abrasion resistance:

Reflectance (color) stability:

Electrical properties:

Radiation resistance:

Flexibility (bend) test:

Adhesion:

Reliability and reproducibility:

Effect on microorganisms:

Comments:

References:

Figure 1. Format for Summary of Information Available on Paint Coatings Applied Externally on Spacecraft - Physical, Chemical and Microbiological Properties

The DDC 1498 data bank and the Science Information Exchange, Smithsonian Institution were queried to identify manufacturers currently active in developing and formulating paint coatings used externally on spacecraft.

These searches resulted in identification of 166 coatings that have been used or evaluated for use on spacecraft. The most commonly used coatings were found to be silicones and silicates. The summarized data resulted in an unscheduled NASA publication*.

Only four coatings of this large group had applicable microbiological information, and this was scant. Three coatings contained fungicides and one other was reportedly "non-nutrient" when in contact with four species of fungi. No supporting microbial exposure data of any type was found.

From the listing of 166 coatings, seventeen were selected for laboratory evaluations with microorganisms. Included in this group were varnishes (4), polyurethanes (3), silicones (2), silicates (2), epoxies (2), acrylics (2), a phenolic-butyrate, and a polyimide. Three of the four varnishes contained fungicides in unspecified amounts. No other coatings were known to contain microbiocides. The coatings are described in more detail in the following section of this report.

PHASE II. MICROBIOLOGICAL EVALUATION

The microbiological evaluations were made by four techniques which take into account Government specification requirements with respect to the resistance of materials to microorganisms; as well as the extractable nutrient or biocidal constituents that may be leached out and concentrated during the random cycles of condensation, accumulation, and drying on materials surfaces.

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* Mayer, R. A., Zaring, M. L., and Kemp, H. T., "Investigation of Spacecraft Coatings", NASA CR-61267 (February, 1969).

Materials and Methods

The paint coatings were obtained directly from the manufacturers or suppliers and applied in the laboratory to 3/8-inch diameter, Iridite-treated 6061-T6 aluminum rods (5 in. long). The rods were radius-rounded to avoid the so-called "edge effect" that occurs on specimens with square edges, i.e., coatings tend to be somewhat thinner at the sharper edges of some types of specimens. Assigned code numbers, identification, and a summary of the data taken during and after preparation of the coated rod specimens are presented in Table 1. As noted in the table, two coatings were applied by the supplier (N-3 and N-17) and two coatings (N-16 and N-17) were applied to non-treated 6060-T6 aluminum rods. All rods were cleaned with methyl ethyl ketone prior to application of the coatings. A fill-and-drain method of application was ordinarily employed although some coatings were sprayed as noted in the table. Although coating procedures were far from being aseptic, an attempt was made to minimize handling and all handling was done with clean white gloves. After curing, the coated rods were inserted into neoprene stoppers, fitted with "breather tubes" and stored in glass bottles as shown in Figure 2. Stoppers and bottles were sterilized before the specimens were mounted. Four specimens were inserted into each stopper. Storage in this manner limited airborne microbial and dust contamination and probably allowed further curing until utilized in the various microbiological experiments. Additional specimens were also included in most of the experiments. These are listed in Table 2.

The rod specimens were cleaned and stored as shown in Table 2 for the coated rods. The honeycomb specimens were mounted singly on the neoprene stoppers by means of 2-inch chrome-plated metal screws.

TABLE 1. SUMMARY OF DATA ON PREPARATION OF COATED ALUMINUM ROD SPECIMENS FOR MICROBIOLOGICAL EVALUATION

Code No.	Coating Type	Viscometer Spindle Number[a]	Viscosity, cps: As Received	Viscosity, cps: After Coating Operation	Cure[b]	Thickness,[c] mils	Hardness[d]: Pencil	Hardness[d]: Rating	Comments
N-1	Varnish (alkyd)	3	130	145	RT	1.0(1)	2B	5	Contained active solvent. Attempts to apply two coats resulted in formation of bubbles and blisters. Varnish contained fungicide.
N-2	Varnish (p-phenyl phenol aldehyde)	3	--	102	RT	0.5(1)	2B	5	Contained active solvent. Attempts to apply two coats resulted in formation of bubbles and blisters. Varnish contained fungicide.
N-3	Li-Al-silicate	--	--	--	--	8.0	2H	10	Applied to rods by supplier. Water sensitive. Conductive coating.
N-4	Polyurethane	3	80	1720	RT	3.0(2)	HB	7	Fungus resistant
N-5	Acrylic	3	560[e]	520	RT	3.0(2)	2B	5	- -
N-6	Acrylic	3	240[e]	330	RT	2.0(2)	B	6	Conductive coating
N-7	Epoxy	3	100	180	RT	3.0(2)	F	8	- -
N-8	Epoxy	3	210[e]	230	RT	4.0(2)	B	6	- -
N-9	Phenolic-butyrate	1	17	19	RT overnight and 45 min. at 250 F	0.5(3)	4H	12	Conductive coating

TABLE 1. (CON'T)

Code No.	Coating Type	Viscometer Spindle Number[a]	Viscosity, cps: As Received	Viscosity, cps: After Coating Operation	Cure[b]	Thickness[c] mils	Hardness[d]: Pencil	Hardness[d]: Rating	Comments
N-10	Varnish (Melamine-alkyd modified)	3	80	84	1st coat 2 hrs at 200F, 2nd coat 5 hrs at 200F	1.5(2)	B	6	--
N-11	Varnish (Melamine-alkyd modified)	3	110	124	(same as above)	1.5(2)	3H	11	Fungicide added
N-12	Polyimide	3	200[e]	200	1st coat 15 min. at 350F, 2nd coat 15 hrs at 350F	0.5(2)	3H	11	--
N-13	Silicone	2	225	325	RT overnight and 90 min. at 450F	2.0(2)	3H	11	--
N-14	Polyurethane	3	200	--	RT	3.5(2)[f]	5H	13	--
N-15	Polyurethane	3	660[e]	740	RT	6.5(2)[f]	HB	7	Conductive coating
N-16	$AlSiO_4$-K_2SiO_2	2	130	135	200F for 1 hr after each coating	7.0(3)	B	6	Applied as spray
N-17	Silicone[g]	--	--	--	RT	10.0	Softer than 6B	< 1	Applied as spray by supplier

(a) Brookfield Model LVT (low viscosity tester) with spindle speed at 60 rpm in all cases.
(b) RT - room temperature
(c) Numbers in parenthesis refers to number of coatings applied.
(d) Pencil hardness was rated by pencil and number, No. 1(6B), soft, to No. 17(9H) hard.

Rating	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pencil	6B	5B	4B	3B	2B	B	HB	F	H	2H	3H	4H	5H	6H	7H	8H	9H

(e) Viscosity adjusted by recommended solvent.
(f) And primer
(g) Applied to nontreated aluminum rods. All others applied to Iridite-treated aluminum rods.

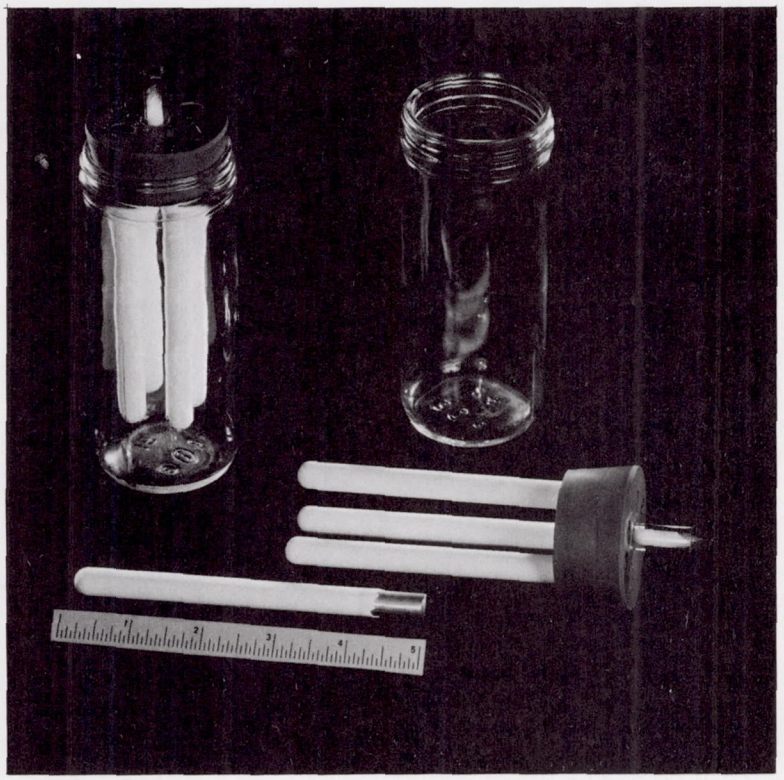

Figure 2. Specimens and Container Jar for Storage and High Humidity Study

TABLE 2. METAL SUBSTRATES AND HONEYCOMBS EVALUATED

Code Number	Type of Specimen
N-18	Stainless steel, Type 304[a]
N-19	6061-T6 aluminum, Iridite-treated[a]
N-20	6061-T6 aluminum[a]
N-21	2024-T3 aluminum sheet, 5052-001 aluminum core[b]
N-22	Prepregnated fiberglass sheet, 5052-001 aluminum core[b]
N-23	Prepregnated fiberglass sheet, heat-resistant phenolic core[b]
N-24	-T3 aluminum sheet, heat-resistant phenolic core[b]

(a) Radius-rounded 3/8-inch diameter, 5 inches long

(b) Honeycombs, 1 in. x 1 in. x 4 in. specimens with sheet on 2 sides and honeycomb core in between

The microbiological procedures employed in these evaluations were as follows:

(1) Extract Studies

a. Water extracts were prepared from all specimens by immersion in sterile distilled water (1×10^6 ohms resistance) and rotary agitation (120 rpm) at 60 C for 96 hours. The area extracted for the coated rods and metal substrates was approximately 100 sq cm per 200 ml of water. The extraction area of the honeycomb materials was unknown. A 2X concentration of the extracts was prepared by immersing extract containers in a mixture of dry ice and acetone until approximately one-half of the extract was frozen. The unfrozen remainder was decanted and evaluated as a 2X concentrate. Sterile NaCl solution was added to those extracts to approximate physiological saline (0.85 percent NaCl). All subsequent dilutions (1:10, 1:100) were made with physiological saline. Determination of the pH of the undiluted (X) extract was made by means of a standard pH meter equipped with a microelectrode.

b. Microbiological evaluations were made individually in test tubes with thrice-washed cells of Pseudomonas aeruginosa, Aspergillus niger, Cladosporium resinae forma avellaneum, and Bacillus subtilis var globigii (vegetative cells). The cells were washed with sterile physiological saline three times by centrifugation, decanting, and resuspending cells in physiological saline. Ten ml aliquots of the extracts were inoculated with sufficient numbers of cells contained in 0.1 ml to result in a final cell concentration of 1000-2000 cells/ml in the 10 ml of extract. Aseptic techniques were used throughout this procedure. Estimates of cell populations in the inocula were made for the bacteria by plate counts of small aliquots while holding the

main volume of inoculum under refrigeration. Fungal cell estimates were made by means of hemocytometer counts. After inoculation, the extracts were incubated at 30 C for varying periods of time and fungal growth rates were determined visually, if possible, or by plate counts if growth could not be seen. Bacterial growth could not be seen and rated visually. Plate counts were therefore conducted on extracts inoculated with Ps. aeruginosa after 4 days of incubation and B. subtilis var globigii after 10 days of incubation. Visual ratings of growth of A. niger and C. resinae forma avellaneum were made during 4 consecutive weeks of incubation. At the conclusion of the 4th week of incubation, all extracts with no apparent fungal growth were plated out to determine whether viable cells of the fungal species had survived.

(2) Petri Plate Studies

Petri plate exposures of coated rods were conducted according to Method 6091 of Federal Test Method Standard No 406* with the following modifications:

a. Large plastic petri plates (150 mm dia.) were used to accommodate the 5-inch long rod specimens.

b. Visual observations were made weekly for 1 month rather than 21 days.

c. A nutrient medium (potato dextrose agar) was used in addition to the specified mineral salts medium.

Briefly, the procedure consisted of spray-inoculating a mixed fungal inoculum of A. niger, A. flavus, Penicillium funiculosum, and Trichoderma sp. on the rod specimens which had been placed on gelled agar surfaces in petri plates. The mixed inoculum was washed three times prior to inoculation with distilled water to minimize

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* Anon. 1961. Plastics: Methods of Testing. Federal Test Method Standard No. 406.

nutrient carryover from stock cultures. A sterile mineral salts solution was used to resuspend the inoculum. Estimates of spore numbers (approximately 1 x 10^6 cells/ml) were made by means of a hemocytometer. Incubation temperature was 30 C for the 4-week incubation period.

(3) High Humidity Evaluation

The technique followed is outlined in MIL-E-5272(ASG) but with the following modifications:

a. The mixed inoculum was prepared and washed in a manner identical to that described above for the petri plate studies.

b. The fungal spore suspension was passed through a double thickness of sterile cotton gauze to remove mycelial fragments.

c. A calibrated spore suspension containing approximately 1 x 10^4 fungal cells was used to spray-inoculate (No. 82 Devilbis atomizer) the 8 specimens of each coating type included in the experiment.

Briefly, the technique involved spraying a mixed inoculum of A. terreus, Memnoniella echinata, Myrothecium verrucaria, and P. citrinum on two sets (8) of rod specimens. The inculated specimens were then placed in jars as shown in Figure 2 and incubated at 30±2 C and 95±6 percent RH for 28 days at which time the specimens were examined for fungal growth.

(4) Soil Burial Exposure

This evaluation was conducted as described in Method 5762 of Federal Specification CCC-T-191b (1951) with no significant modifications and two evaluation procedures to determine coating changes, namely, pencil hardness and electrical resistance measurements. Pencil hardness was determined with a series of drawing pencils of

graded hardness (see Table 1) by scratching a coating surface until the hardest pencil not penetrating the coating was found. This procedure was satisfactory for most coatings, but N-17 (a silicone) was too soft to be measured in this manner. The electrical resistance measurement was taken by means of a Model 602 Electrometer with two alligator clip-wire leads, one to the bare end of the coated aluminum rod under test and the other to the side of the stainless steel container in which the soil bed was contained. Pencil hardness and electrical resistance measurements were taken initially and after 1, 2, 3, 4, and 6 months of exposure. Notes on visible changes of the coatings were also taken during the exposure period.

Scanning electron microscopy (SEM) was employed to examine those coatings which had softened after soil burial exposure or which had significant loss of electrical resistance after 4 to 6 months of exposure. One set of each type of coated rods was also subjected to cleaning or "cavitation erosion" by exposure to fresh tap water at 22 kH for 15 seconds at 39 C in a Blackstone Model 1.9 ultrasound cleaner. Appropriate nonexposured and ultrasound-cleaned control specimens were also examined for comparison.

The microorganisms used in these studies were as follows:

Organism	Code No.[a]
Aspergillus flavus	QM 380
Aspergillus niger	QM 386
Aspergillus terreus	ATCC 10690 (QM-82-J)
Bacillus subtilis var *niger*	BMI-2
Cladosporium resinae forma *avellaneum*	BMI-29
Clostridum sporogenes	ATCC 11437

Organism	Code No[(a)]
Memnoniella echinata	ATCC 9597
Myrothecium verrucaria	ATCC 9095
Penicillium citrinum	ATCC 9840
Penicillium funiculosum	QM 391
Pseudomonas aeruginosa	BMI-37
Trichoderma sp	QM 365

(a) Sources were QM = U. S. Army Quartermaster, Natick, Massachusetts;
ATCC = American Type Culture Collection, Rockville, Maryland;
BMI = Battelle Memorial Institute Culture Collection

Stock cultures of the fungi were grown on test tube slants of potato dextrose agar (Difco). Except for Cl. sporogenes which was cultured in thioglycolate broth (Difco), the bacteria was cultured on test tube slants of tryptone glucose extract agar (Difco) supplemented with 0.5 percent yeast extract. All were maintained at 30 C.

Several microbiological studies were conducted to determine whether microbial contaminants occurred in umpolymerized "from-the-container" materials and in water extracts of the cured coatings. Standard procedures involving appropriate agar media, glass "hockey stick" spreaders, incubation temperature (30 C) and times (up to 28 days) were employed.

The standard plating procedures were also used in recovering spores of B. subtilis var niger and Cl. sporogenes from coating and substrate surfaces which had been spray inoculated with thrice-washed spores of these bacteria and incubated anaerobically in a hydrogen atmosphere at 30 C. A methylene blue indicator was used in the chamber to assure the anaerobic condition. The inocula

contained approximately 1 X 10^6 spores/ml. Each rod specimen was sprayed with approximately 0.05 ml of spore inoculum. Recovery of spores was accomplished after incubation (up to 48 days) with sterile cotton swabs which were placed in sterile physiological saline, agitated by means of a Vortex mixer, and spreading 0.1 ml aliquots of 1:10, 1:100, and 1:1000 dilutions on the surface of the tryptone agar medium described above. Incubation temperature was 30 C for both organisms.

Results

"From-the-container" studies were initiated to determine whether unpolymerized coatings or coating components contained viable microorganisms. The results of this study are presented in Table 3. In no case were microoganisms consistently found in any of the coatings or coating components evaluated. If microorganisms are contaminants in any of these coatings prior to curing, then the number must be quite low, and a much greater sampling of each polymer must be undertaken to determine approximate numbers and types. No more than two storage containers were available for this sampling. This is not adequate for reliable sampling. The coatings N-3 and N-17 were applied to rod specimens by suppliers and unpolymerized material was not available for study.

Following extraction, water extracts were refrigerated (5 C) until used in laboratory evaluations. A number of the coating extracts (N-5, N-9, N-10, N-11, N-12, N-14, N-15, N-16, and N-17) contained obvious contaminating microbial growth despite attempts to maintain aseptic handling procedures prior to this. Three honeycomb extracts (N-21, N-23, and N-24) also contained contaminant growth. None of the metal substrate extracts contained growth. Although fungi predominated

TABLE 3. ESTIMATES OF NUMBERS OF MICROORGANISMS IN UNPOLYMERIZED COATINGS FROM STORAGE CONTAINERS

Battelle Code No.[a]	Dilution, Growth Observed[b]		
	Undiluted	1:100	1:1000
N-1	- (0/4)	± (2/4)	± (3/4)
N-2	- (0/4)	± (1/4)	± (2/4)
N-4A	± (1/4)	- (0/4)	± (1/4)
N-4B	± (2/4)	± (1/4)	± (2/4)
N-5	± (1/4)	± (2/4)	± (2/4)[c]
N-6	± (2/4)[c]	± (3/4)	± (1/2)
N-7A	± (2/4)[c]	± (1/4)	± (3/4)[c]
N-7B	± (1/4)[c]	± (2/4)	± (2/4)[c]
N-8A	- (0/4)	± (2/4)	± (1/4)
N-8B	- (0/4)	± (2/4)	± (1/4)
N-9	± (1/4)	- (0/4)	- (0.4)
N-10	- (0/4)	± (1/4)	- (0.4)
N-11	- (0/4)	± (1/4)	- (0/4)
N-12	± (2/4)	± (1/4)	- (0/4)
N-13	- (0/4)	± (1/4)[c]	± (2/4)
N-14A	± (1/4)	± (1/4)	± (2/4)
N-14B	- (0/4)	- (0/4)	± (1/4)
N-15A	± (1/4)	± (1/4)	± (3/4)[c]
N-15B	± (1/4)	- (0/4)	- (0/4)
N-16	± (2/4)	- (0/4)	± (2/4)

(a) Coatings N-3 and N-17 were not evaluated. These coatings were applied by the respective suppliers and nonpolymerized material was not available.

(b) Ratings for growth were: - = no organisms
± = few organisms
+ = consistent presence of organisms (none occurred).

The numbers in parenthesis indicate the number of petri plates with organisms/number of plates evaluated, for example, 0/4 indicates that no organisms were observed on four plates.

(c) Organisms too numerous to count occurred on one of four plates.

among the contaminants, bacteria were also evident in some cases. Microbial growth under these conditions (5 C and minimal available nutrients) is somewhat unusual and is a good indication that the coatings and honeycomb materials could be improved in regard to limiting microbial nutrient release from them.

Results of the water extract evaluations are presented in Table 4. Of the four microorganisms evaluated, A. niger grew most profusely and utilized a wider range of extracts than any other. B. subtilis (vegetative cells) was the most sensitive, i.e., grew or survived in the fewest number of extracts.

Coating extracts that particularly stimulated growth of one or more of the microorganisms were:

N-4 (polyurethane)
N-5 (acrylic)
N-6 (acrylic)
N-7 (epoxy)
N-8 (epoxy)
N-9 (phenolic-butyrate)
N-10 (varnish)
N-12 (polyimide)
N-13 (silicone)
N-14 (polyurethane)
N-15 (polyurethane)
N-16 ($AlSiO_4$-K_2SiO_2)
N-17 (silicone)

None of the remaining coating extracts inhibited the growth of all four species, i.e., at least two species grew or survived in these extracts.

Water extracts of the metal substrates, N-18 (stainless steel) and N-20 (aluminum), apparently stimulated growth of most of the test species, while only slight growth or survival was noted in the extract of N-19 (Iridite-treated aluminum). The latter aluminum substrate appears to be superior in this regard to the other metals.

TABLE 4. SUMMARY OF GROWTH OF INDIVIDUAL BACTERIA AND FUNGI IN WATER EXTRACTS FROM COATING, SUBSTRATE, AND HONEYCOMB SPECIMENS

Specimen Code No.	pH[a]	Growth Rating at Indicated Dilution[b]														
		Pseudomonas aeruginosa				Aspergillus niger				Cladosporium resinae			Bacillus subtilis			
		2X	X	1:10	1:100	2X	X	1:10	1:100	X	1:10	1:100	X	1:10	1:100	
N-1	4.7	-	-	-	-	-	-	+	+	-*	-*	-	-	-	-	
N-2	5.6	-	-	-	-	-*	-*	±	+	-*	-*	-	-	-	-	
N-3	8.9	-	-	+	+	-*	-*	±	±	-	-	-*	-	-	-	
N-4	7.4	-	±	-	++	++	++	±	-*	-	-*	-*	-	-	-	
N-5	6.5	-	-	-	±	++	++	+	±	-	-	-*	+	-	-	
N-6	6.4	-	±	±	±	±	++	++	+	-	-	-*	+	++	-	
N-7	7.1	++	++	++	-	++	++	+	+	+	-*	-*	-	-	-	
N-8	7.1	+	++	+	±	++	+	+	±	±	-*	-*	-	-	-	
N-9	7.0	-	-	-	-	++	++	+	-*	-	-*	-*	-	-	-	
N-10	6.2	-	-	-	-	++	++	+	±	-	-*	-*	-	-	-	
N-11	5.0	-	-	-	-	-	-	-	+	-	-	-*	-	-	-	
N-12	6.3	-	-	-	-	++	++	+	±	+	-*	-*	-	-	-	
N-13	7.3	-	+	-	-	++	++	±	-*	+	-*	-*	-	-	-	
N-14	6.5	++	-	-	±	++	++	+	±	-	-	-	++	-	-	
N-15	5.9	-	-	-	-	++	++	+	±	++	+	-*	-	-	-	
N-16	5.2	-	-	-	-	+	±	±	-*	+	-*	-*	+	-	-	
N-17	7.8	++	++	++	++	-*	++	-*	-*	+	+	-	++	-	-	
N-18	7.2	++	±	-	++	++	++	+	-*	±	-*	-*	++	-	-	
N-19	6.4	-	±	-	±	-*	-*	-*	-*	±	-*	-*	-	-	-	

TABLE 4. (CON'T)

Specimen Code No.	pH[a]	Growth Rating at Indicated Dilution[b]														
		Pseudomonas aeruginosa				Aspergillus niger				Cladosporium resinae			Bacillus subtilis			
		2X	X	1:10	1:100	2X	X	1:10	1:100	X	1:10	1:100	X	1:10	1:100	
N-20	6.8	+	±	±	++	+	++	+	-*	+	-*	-	++	-	-	
N-21	7.2	++	-	-	-	-*	++	-*	-*	Not evaluated			++	++	++	
N-22	7.1	-	-	±	-	+	+	+	-*	Not evaluated			Not evaluated			
N-23	7.2	±	-	-	-	++	+	+	-*	-*	-	-*	+	+	+	
N-24	7.1	-	-	-	±	++	+	+	-*	-	-*	-*	++	-	-	
Control[c]	7.1		+			-*				-*			-			

(a) Specimens were extracted in distilled water (pH - 7.12) by rotary shaking (120 rpm) at 60 C for 96 hours. The 2X dilutions were prepared by freeze-concentration. Determination of pH was made with standard pH meter with microelectrode. Sodium chloride was added to approximate physiological saline.

(b) Growth ratings were:

- = None
± = Slight growth (less than control)
\+ = Growth about same as control
++ = Growth greater than control
* = Fungal growth observed after incubation

Ratings of bacterial growth were based on plate counts after incubation. Fungal growth in test tubes was rated visually. Asterisks(*) indicate recovery of vis ble fungal cells after the incubation period. Each tube was inoculated with enough washed cells to result in a final concentration of approximately 1000 cells/ml.

(c) Controls consisted of sterile saline prepared with distilled water.

Water extracts of the honeycomb specimens N-21, N-23, and N-24 stimulated growth of at least one of the test species, while the N-23 extract allowed minimal growth of the two species evaluated.

Water extractable materials were apparently obtained from all specimens. The extract pH determinations indicate that an undetermined amount of extraction occurred under the conditions employed, i.e., extractables from most specimens significantly altered the pH of the distilled water (pH 7.12) extraction medium. When pH was unchanged by extractables, the growth of one or more test organisms was stimulated, indicating the presence of extractable nutrients or stimulatory substances.

The results of the petri plate studies are presented in Table 5. The specification procedure followed calls for the use of mineral salts agar. In such an evaluation, inclusion of a nutrient agar medium is useful for comparing the degree of fungal growth in the presence of an additional nutrient source. In this experiment, coatings N-7 (epoxy), N-10 (varnish), N-14 (polyurethane), and N-15 (polyurethane) supported moderate to heavy fungal growth on both media; while coatings N-4 (polyurethane), N-5 (acrylic), N-6 (silicate),and N-17 (silicone) supported moderate to heavy growth only on potato dextrose agar. Several coatings had no growth or very slight growth when placed on mineral salts agar and for this reason performed best in this evaluation. These were coatings N-1 (varnish), N-2 (varnish), and N-3 (silicate). No zones of inhibition were noted around any of the specimens on either medium.

Of the metal substrates, only Iridite-treated aluminum inhibited or limited the growth of the microorganisms. The honeycomb specimens N-21 and N-24 had no

TABLE 5. GROWTH OF MIXED FUNGAL INOCULUM ON SPECIMEN SURFACES IN PETRIPLATE EXPOSURE AT 30 C AND 95 ± 5% RH

Specimen Code No.	Growth Rating[a]	
	Mineral Salts Agar[b]	Potato Dextrose Agar[c]
N-1	±	+
N-2	±	+
N-3	-	+
N-4	+	++
N-5	-	++
N-6	+	+++
N-7	++	+++
N-8	+	+
N-9	+	++
N-10	+++	+++
N-11	+	+
N-12	-	++
N-13	-	++
N-14	++	+
N-15	++	++
N-16	+	++
N-17	+	++
N-18	+	++
N-19	-	+

TABLE 5. (CON'T)

Specimen Code No.	Growth Rating[a] Mineral Salts Agar [b]	Potato Dextrose Agar [c]
N-20	+	++
N-21	-	±[e]
N-22	-	+[e]
N-23	-	+[e]
N-24	-	++[e]
Control[d]	++	+++

(a) Growth ratings are:

- = None

± = Questionable or very slight

+ = slight

++ = moderate

+++ = heavy

(b) Non-nutrient agar medium (c) Nutrient agar medium

(d) Plates with indicated media and sterile squares of filter paper sprayed to ascertain viability of inoculum

(e) Heavy bacterial contamination probably from handling. Pit corrosion also noted, especially on the 5052-001 aluminum core material.

growth on mineral salts agar but slight to moderate growth on potato dextrose agar. Honeycomb N-24 supported the most growth. Photographs of typical fungal growth on selected coatings are shown in Figures 3 through 5.

In this study, a leached (running water bath at 30 C for 18 hours with water flow at 10 l/hr) series of specimens was included. The results were essentially the same as those reported in Table 5.

The results of the high humidity study are shown in Table 6. Moderate fungal growth occurred on only two specimens, namely N-4 (polyurethane) and N-10 (varnish). Slight or questionable growth occurred on N-3, N-6, N-11, N-12, N-13, N-14, N-16, and N-17.

However, N-4 (polyurethane), N-8 (epoxy), N-15 (polyurethane), and N-16 (silicate) softened at least two pencil ratings during the exposure period. The softening appeared to be associated with microbial growth for N-4 and N-16; while N-8 and N-15 apparently softened because of the high humidity, i.e., no microbial growth was evident on these specimens as determined by microscopic examination.

Results of soil burial exposure of the coated specimens are summarized in Table 7.

During 6 months of exposure the following coatings softened four or more pencil hardness ratings: N-3 (silicate), N-4 (polyurethane), N-8 (epoxy), N-15 (polyurethane), and N-16 (silicate)

Coatings that lost at least two pencil hardness ratings were: N-1 (varnish), N-11 (varnish), N-13 (silicone), and N-14 (polyurethane).

All other coatings did not soften or softened only one pencil hardness rating which was considered insignificant. This method was not applicable to N-17 (silicone) because it was too soft to measure.

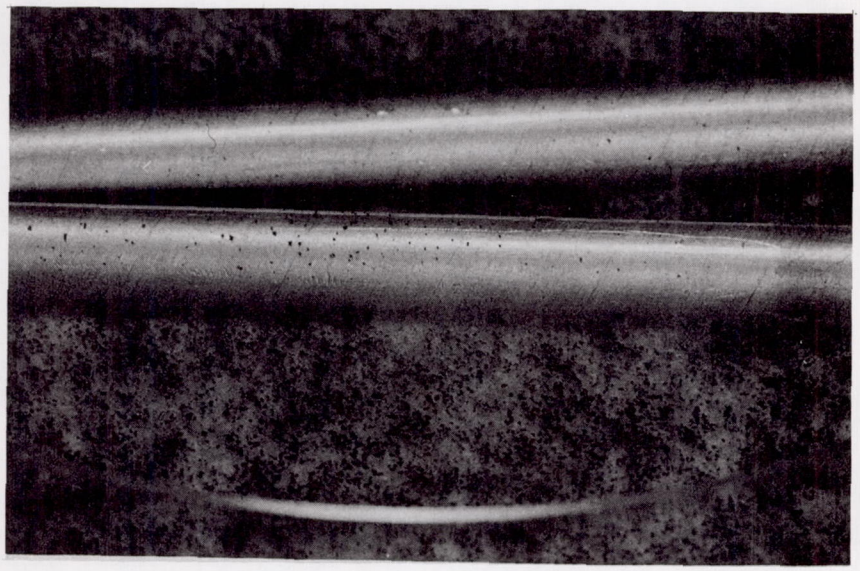

Figure 3. Typical Fungal Growth on Coating N-10 (Varnish) in Petri Plate Study on Potato Dextrose Agar

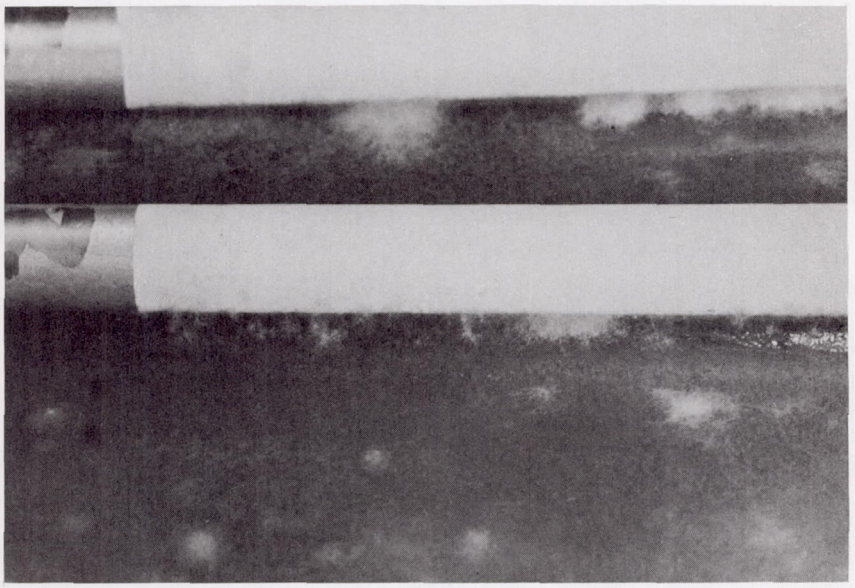

Figure 4. Typical Fungal Growth on Coating N-14 (Polyurethane) in Petri Plate Study on Mineral Salts Medium

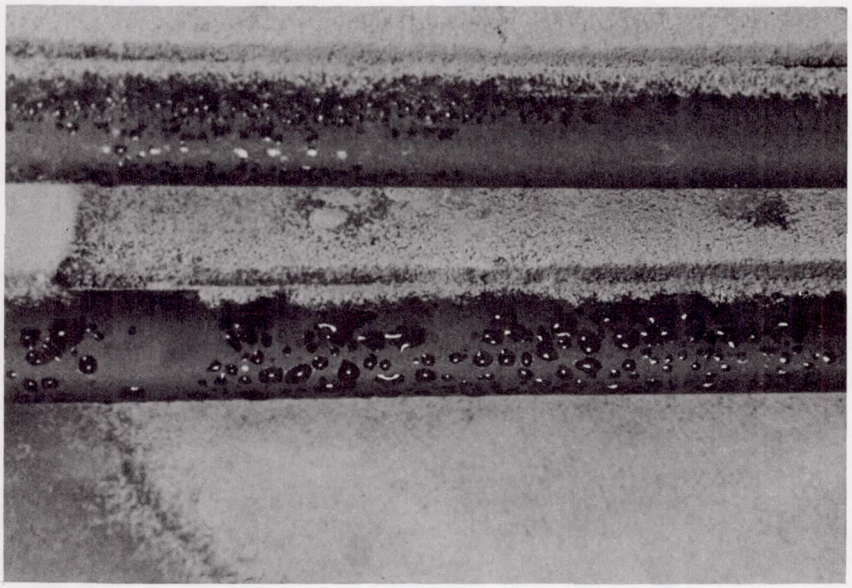

Figure 5. Typical Fungal Growth on Coating N-6 (Acrylic) in Petri Plate Study on Potato Dextrose Agar

TABLE 6. NOTES ON GROWTH OBSERVED MICROSCOPICALLY ON SPECIMENS INOCULATED WITH MIXED FUNGAL INOCULUM AFTER 30 DAYS OF INCUBATION AT 30 C AND 95 ± 5% RH

Specimen Code No.	Observation	Growth Rating[a]
N-1	No growth, spores not germinated	-
N-2	No growth, spores not germinated	-
N-3	Spores germinated, scant mycelial development	+
N-4	Spores germinated, slight-moderate mycelial development	++
N-5	No growth, spores not germinated	-
N-6	Spores germinated, scant mycelial development	+
N-7	No growth, spores not germinated	-
N-8	No growth, spores not germinated	-
N-9	No growth, spores not germinated	-
N-10	Spores germinated, slight to moderate mycelial development	++
N-11	Growth on 3 of 8 rods, scant mycelial development	±
N-12	Spores germinated, scant mycelial development	+
N-13	Germinated spores on 4 of 8 rods	±
N-14	Spores germinated, scant mycelial development	+
N-15	No growth	-
N-16	Spores germinated, scant mycelial development	+

TABLE 6. Continued

Specimen Code No.	Observation	Growth Rating[a]
N-17	Spores germinated, scant mycelial development	+
N-18	No growth or spore germination	-
N-19	No growth or spore germination	-
N-20	Growth on 1 of 8 rods, scant mycelial development	±
N-21	No growth or spore germination[b]	-
N-22	No growth or spore germination[b]	-
N-23	Occasional spores germinated	±
N-24	No growth or spore germination[b]	-

(a) Growth ratings were:

- = No growth or spore germination

± = Questionable or partial spore germination or growth

\+ = Definite but scant mycelial development

++ = Slight moderate mycelial development

(b) Pit corrosion of aluminum noted, especially on the 5052-001 aluminum core material.

TABLE 7. MEASUREMENT OF COATING PROPERTIES DURING SOIL BURIAL EXPOSURE

Coating Code No.	Measurement at Time Noted, months: Pencil Hardness(a) Initial	1	3	6	Electrical Resistance, ohms(b) Initial	1	3	6
N-1	8	7	7	6	$1.2X10^7$	$1.8X10^7$	$9.4X10^6$	$3.2X10^6$
N-2	7	8	8	6	$3.1X10^7$	$7.9X10^6$	$5.6X10^6$	$3.2X10^6$
N-3	10	6	6	6	Not applicable - Conductive coating			
N-4	11	1	1	2	$> 1.0X10^{12}$	$1.3X10^{10}$	$3.2X10^{10}$	$5.1X10^9$
N-5	8	6	7	7	$2.7X10^7$	$5.2X10^5$	$3.0X10^5$	$2.7X10^5$
N-6	9	8	8	9	$1.4X10^7$	$8.0X10^6$	$0.5X10^5$	$0.7X10^5$
N-7	9	9	8	8	$1.9X10^9$	$2.9X10^8$	$6.4X10^7$	$3.9X10^6$
N-8	11	4	8	7	$2.3X10^6$	$3.1X10^5$	$1.6X10^5$	$1.2X10^5$
N-9	11	8	10	11	Not applicable - Conductive coating			
N-10	5	5	5	5	$1.5X10^5$	$1.2X10^7$	$1.2X10^6$	$2.8X10^5$
N-11	11	9	8	9	$6.0X10^7$	$1.0X10^8$	$6.5X10^7$	$3.1X10^7$
N-12	11	11	11	11	Not applicable - Conductive coating			
N-13	11	9	9	9	Not applicable - Conductive coating			
N-14	13	12	13	11	$2.7X10^8$	$1.7X10^8$	$2.8X10^8$	$3.1X10^8$
N-15	13	10	11	9	Not applicable - Conductive coating			
N-16	6	3	2	2	Not applicable - Conductive coating			
N-17	< 1	< 1	< 1	< 1	$2.7X10^8$	$1.5X10^5$	$1.6X10^5$	$1.5X10^5$

(a) See footnote (d) at end of Table 1. Initial pencil hardness readings are different in some cases than those presented in Table 1. This is probably due to additional curing during storage at room temperature until soil burial was started.

(b) Measurement made in situ with Model 602 Electrometer. Uncoated substrates (aluminum and stainless steel) varied between 0.5 - 1.1X105 ohms during the exposure period. Conductive coatings also had measurements in this approximate range.

Losses in electrical resistance in the range of 2 to 3 logs were recorded for: N-4 (polyurethane), N-5 (acrylic), N-6 (acrylic) N-7 (epoxy) and N-17 (silicone).

One log loss in electrical resistance occurred for: N-1 (varnish), N-2 (varnish) and N-8 (epoxy).

Only minor losses in electrical resistance occurred for the remainder of the coatings, which appear to be somewhat resistant to microbial degradation. This method of measuring coating change was not suitable for conductive coatings (N-3, N-9, N-12, N-13, N-15, and N-16).

There was no apparent correlation between softening as determined by pencil hardness change and loss in electrical resistance. If a coating exhibited a loss in either property, then deterioration apparently occurred. As noted, the deterioration may have been caused by either microbial action or by the humid or wet conditions of the experiment and the resulting water absorption by the coating. In either case, 9 of the 17 coatings were severely affected as determined by one or the other evaluation procedures as noted above.

Scanning electron microscope (SEM) examination was employed to determine whether changes in surface features could be observed for selected specimens after soil burial exposure. In a preliminary study of control, exposed, and ultrasound-cleaned specimens of coating N-17 (silicone) after 4 months of soil burial, the SEM photomicrographs shown in Figures 6 through 9 were obtained. The following remarks can be made:

Figure 6: The non-exposed (control) surface of silicone coating N-17 is quite rough with pigment particles of less than 1 μ and deep irregular pits greater than 1 μ in diameter.

Figure 7: Moderate damage to the surface of this coating occurred in localized areas with apparent loss of pigment particles. Some areas of this coating surface were similar to that shown in Figure 6.

Figure 8: The surface of the control specimen after ultrasound cleaning was similar to the non-exposed control surface shown in Figure 6, indicating that the ultrasound cleaning alone apparently did not affect this coating surface.

Figure 9: This coating surface is entirely different from any of the previous surfaces shown. It is relatively smooth with small microfissures throughout. Apparently soil burial exposure essentially softened or decomposed the matrix allowing the pigment particles to break loose during the cleaning process. This, along with the previously-reported loss of electrical resistance after soil burial, provides a strong indication that this coating is susceptible to microbial deterioration.

SEM examination at magnifications between 100 and 10,000X of selected coatings after 6 months of soil burial may be briefly summarized as follows:

N-3 (silicate) - Fissures (up to 50 μ width) were obvious in this coating surface at 100X in all specimens examined, including the non-exposed control. The fissures were somewhat larger and occurred more frequently in specimens exposed to soil burial and ultrasound cleaning. At 2000X, the surface of all specimens was quite rough with irregularly shaped pits up to 3 μ in diameter.

N-4 (polyurethane) - The coating surface of all specimens was very smooth at all magnifications to 10,000X. Occasional microfissures (0.1 μ width) were observed

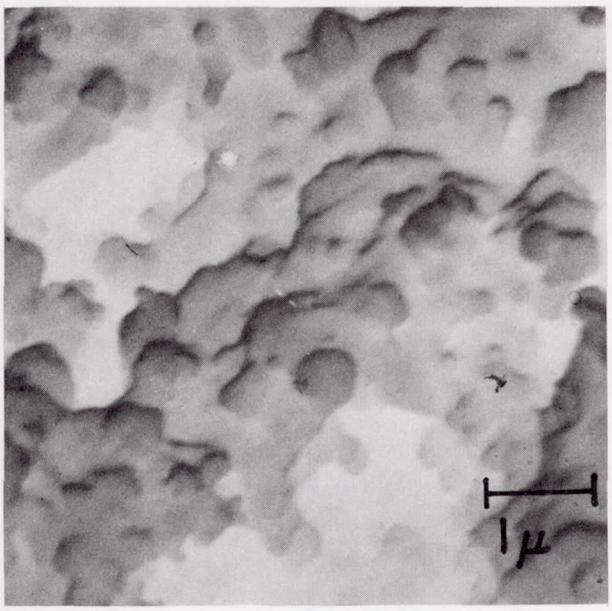

Figure 6. Control (Non-Exposed) Surface of Coating N-17 (Silicone): SEM - 10,000X

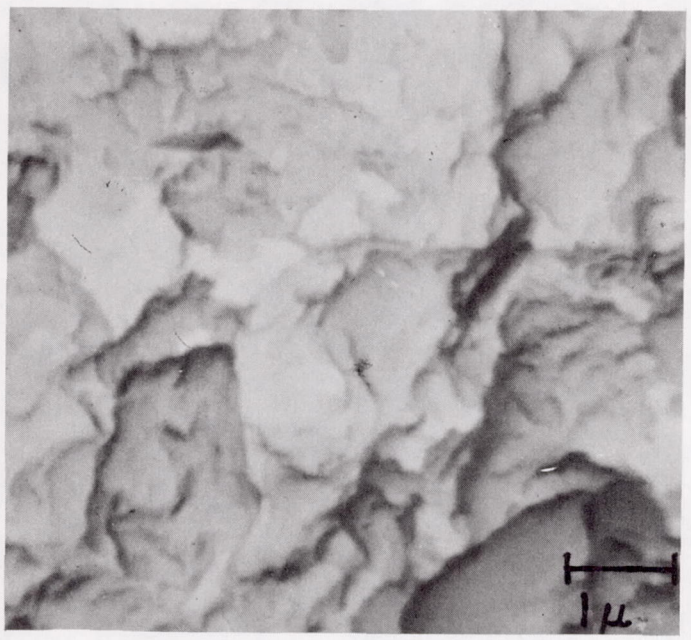

Figure 7. Surface of Coating N-17 After 4 Months of Soil Burial Exposure: SEM - 10,000X

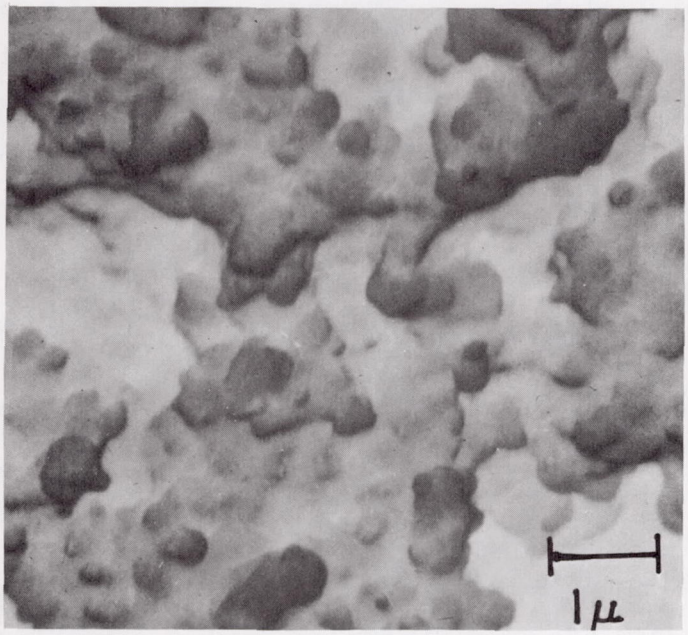

Figure 8. Control (Non-Exposed) Surface of Coating N-17 After Ultrasound Cleaning: SEM - 10,000X

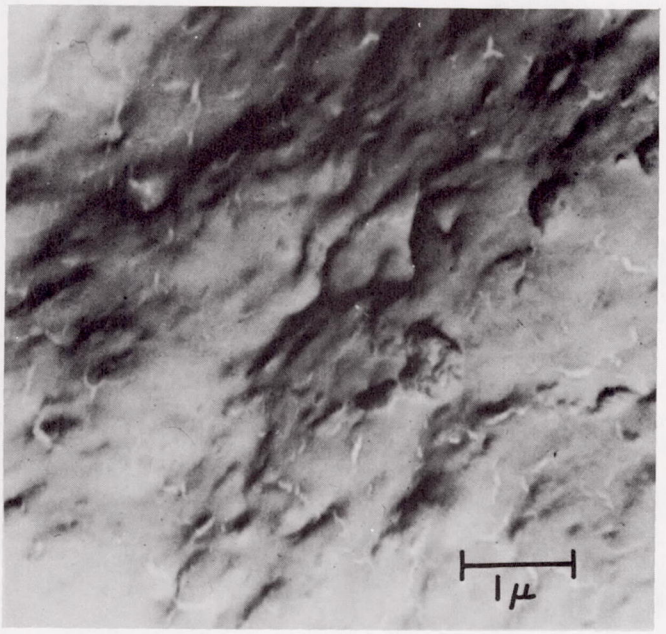

Figure 9. Surface of Coating N-17 After 4 Months of Soil Burial Exposure and Ultrasound Cleaning: SEM - 10,000X

in all specimens except non-exposed controls. The microfissures were more frequent in the specimen exposed to soil burial and ultrasound cleaning. Greater damage than this was expected since this coating was one of the more severely damaged in soil burial as determined by losses in pencil hardness and electrical resistance.

N-5 (acrylic) - Surfaces of all coating specimens were moderately smooth with bumps due apparently to underlying pigment particles. Microfissures were rarely observed in all specimens whether exposed or not. Since no evidence of physical damage of the coating surface was observed, chemical changes in the coating due to soil burial exposure are suspected.

N-6 (acrylic) - Moderately smooth coating surfaces for all specimens were observed at magnifications up to 500X although pigment particles and air bubbles caused some surface irregularity. Some air bubbles were partially ruptured with essentially the appearance of pinholes in all specimens examined. Soil burial exposure apparently caused more frequent and severe rupturing of air bubbles (observed at 2000X) and may be the reason for loss of electrical resistance.

N-8 (epoxy) - Surfaces of this coating were very smooth at magnifications up to 2000X for non-exposed, soil-burial-exposed, and ultrasound cleaned specimens. However, the surface of the specimen from soil burial and cleaned with ultrasound was quite rough with irregularly shaped pigment particles (approximately 5 μ long x 2-3 μ wide). The combination of soil burial exposure and ultrasound cleaning apparently removed some of the polymer matrix. Pinholes were occasionally observed in all specimens.

N-10 (varnish) - The surfaces of all specimens of this coating were very smooth at magnifications up to 2000X. Very small microfissures (<0.1 μ) were observed at 10,000X on surfaces exposed to soil burial alone and to soil burial with

ultrasound cleaning. No microfissures were evident on nonexposed or ultrasound-cleaned surfaces. This coating performed well in soil burial with no loss of hardness or electrical resistance although it did support or stimulate microbial growth in other studies.

N-12 (polyimide) - The surfaces of all specimens were exceptionally smooth at magnifications up to 2000X. Pinholes were rarely observed in all specimens whether exposed to soil burial or not: The pinholes appeared to be formed by air bubbles which had collapsed and filled in. There was no real evidence of any physical differences in these specimens. This coating performed well in soil burial with no loss in hardness although extracts stimulated the growth of two fungi.

N-14 (polyurethane) - Surfaces of this coating were moderately smooth with some irregularity due to pigment particles. Fissures (some up to 50 μ width) similar to scratches were observed on all specimens. Pinholes (approximately 5 μ) were also found occasionally. Enlargement of the fissures occurred on specimens exposed to soil burial alone and cleaned by ultrasound.

N-16 (silicate) - The surfaces of all specimens were very rough with a porous texture (observed at 2000X). There were no apparent changes due to soil burial exposure or ultrasound treatment. Chemical changes in this coating are apparently involved in the significant loss of coating hardness during soil burial.

N-17 (silicone) - The series of SEM photomicrographs for this coating after 6 months of soil burial were essentially similar to those obtained after 4 months of soil burial (Figures 6 through 9). These photomicrographs were previously discussed.

For the purposes of minimizing microbial contamination and entry points into the matrix of a coating, smooth-surfaced coatings are preferable to ones with rough surfaces. Pinholes and fissures must be minimal for the same reasons. Of the coatings examined by SEM, N-4 (polyurethane), N-8 (epoxy), N-10 (varnish), and N-12 (polyimide) without question had the smoothest surfaces. Microfissures, pinholes, or coating change after soil burial exposure occurred for three of these coatings (N-4, N-8, and N-10) so that coating N-12 appears to be superior from this point of view. Coatings N-3 (silicate), N-16 (silicate), and N-17 (silicone) were quite rough initially and changed significantly after soil burial exposure. Coatings N-5 (acrylic), and N-14 (polyurethane) were intermediate in smoothness with only N-5 remaining apparently unchanged after soil burial.

The data obtained from bacterial spore studies are summarized in Table 8. Greater survival was noted for Cl. sporogenes spores than B. subtilis, although the latter survived surprisingly well under the anaerobic conditions of this experiment. As might be expected, B. subtilis spores were hardier than the vegetative cells of this organism in the previously described extract study (Table 4). One coating, N-3 (silicate), totally inhibited spores of both species while several others (N-10 varnish, N-11 varnish, and N-14 polyurethane) totally inhibited only B. subtilis spores. Strong inhibition of spores of both bacterial species occurred with N-1 (varnish) and N-17 (silicone). Some specimens appeared to enhance spore survival of both species. These were N-12 (polyimide), N-15 (polyurethane), N-16 (silicate), and the honeycomb materials, N-22 and N-23. Other specimens enhanced spore survival of one of the species namely, N-2 (varnish), N-4 (polyurethane), N-5 (acrylic), N-6 (acrylic), N-7 (epoxy), N-10 (varnish) N-14 (polyurethane), and the honeycombs, N-21 and N-24. For all other specimens (N-8, N-9, and N-13), the spore recovery rates were essentially the same as those obtained for the metal substrates (N-18, N-19, and N-20), i.e., within the range of 1000-6000 spores/cm^2 recovered.

TABLE 8. RECOVERY OF BACTERIAL SPORES FROM SPECIMEN SURFACES AFTER ANAEROBIC INCUBATION AT 30 C

Specimen Code No.	Spore Recovery Rating[a]	
	B. subtilis var niger[b]	*Clostridium* sporogenes[c]
1	±	±
2	+	++
3	-	-
4	+	++
5	++	+
6	+	++
7	+	++
8	+	+
9	+	+
10	-	++
11	-	--[d]
12	++	++
13	+	+
14	-	++
15	++	++
16	++	++
17	±	±
18	+	+

TABLE 8. (CON'T)

Specimen Code No.	Spore Recovery Rating[a]	
	B. subtilis[b] var niger	*Clostridium*[c] sporogenes
19	+	+
20	+	+
21	+	++
22	++	++
23	++	++
24	±	++

(a) Recovery of spores from specimen surfaces was accomplished with sterile cotton swabs which were agitated in sterile physiological saline. The resulting spore-containing suspension was serially diluted and plated out on tryptone glucose extract agar (supplemented with 0.5 percent yeast extract). The plates were then incubated in a hydrogen atmosphere for varied periods of time and the resulting colonies counted. The rating system is as follows:

- \- = no recovery
- ± = 1 - 1000 cells/cm^2 recovered
- \+ = 1000 - 6000 cells/cm^2
- ++ = more than 6000 cells/cm^2

(b) Incubated 37 days

(c) Incubated 48 days

(d) Not included

These results could not have been predicted from growth inhibition of microorganisms in previously-described studies in this report. For example, the spore kill that occurred with N-3 (silicate) may have been brought about by a high pH since a pH of 8.9 was obtained for the water extract of this coating. The fairly strong spore inhibition by N-17 (silicone) was certainly not expected since vegetative microbial forms in all other exposure studies either were stimulated or at best survived in the presence of this coating or extracts from it. However, on the basis of strong inhibition or kill of both spore forms, the coatings N-1 (varnish), N-3 (silicate), and N-17 (silicone) performed best in this study. Coating N-11 (varnish) may be as good but data were obtained only for B. subtilis.

SUMMARY AND CONCLUSIONS

On the basis of all the evaluations conducted, none of the coatings inhibited microbial growth totally. Therefore, none of the coatings as they are now formulated are suitable from a spacecraft sterilization viewpoint without terminal or some other sterilization process.

Some of the coatings are fairly resistant to microbial degradation as judged by minimal or no changes in pencil hardness or electrical resistance in soil burial exposure and minimal or slight microbial growth in most of the other types of exposures. These are:

N-2 (varnish)

N-9 (phenol-butyrate)

N-12 (polyimide)

All other coatings significantly softened, lost electrical resistance, or had moderate to heavy growth in more than one type of evaluation; and could be improved in regard to resistance to microbial attack.

Three varnishes (N-1, N-2, and N-11) contained fungicides. One (N-10) did not. As a class, varnishes were less susceptible to microbial attack and supported less microbial growth than other coatings. The non-fungicide-containing-varnish (N-10) stimulated the growth of more microorganisms than any of the other varnishes although it performed well during soil burial exposure.

The polyurethanes (N-4, N-14, and N-15) appear to be the class of coating most susceptible to microbial attack with N-14 and N-15 performing somewhat better than N-4.

The two acrylics, N-5 and N-6, appeared to be somewhat resistant to degradation but supported the growth of many of the microorganisms and lost two logs electrical resistance during soil burial. Of the two, N-5 appears to be the better coating on the basis of less microbial growth in petri plate and high humidity evaluations.

Both epoxy coatings (N-7 and N-8) evaluated supported the growth of most of the microorganisms. Pencil hardness rating losses in high humidity and soil burial exposures occurred for N-8, and epoxy N-7 appears preferable.

The silicate coatings N-3 and N-16 softened significantly during soil burial although neither markedly stimulated microbial growth in other evaluations. Coating N-3 is known to be sensitive to water over prolonged exposure periods. This may have been the reason for the loss of hardness recorded. The same may be true for the N-16 silicate coating.

Although both can provide nutrients for microbial growth and survival, the phenolic-butyrate coating (N-9) and the polyimide coating (N-12) appear to be resistant to microbial attack as judged by lack of changes during soil burial.

Scanning electron microscope examination revealed the surface features of selected coatings as well as resulting damage from soil burial exposure for some samples. Enlargement or occurrence of pinholes, fissures, microfissures or other changes due to soil burial ultrasound cleaning were observed for coatings N-3 (silicate), N-4 (polyurethane), N-6 (acrylic), N-8 (epoxy), N-10 (varnish), N-14 (polyurethane), and N-17 (silicone). The damage observed did not always coincide with measured losses in soil burial exposure, e.g., N-10 did not soften or lose electrical resistance although it did support microbial growth in extract, petri plate, and high humidity studies. Examination of coatings N-5 (acrylic)

and N-16 (silicate) revealed no obvious damage to coating surfaces, and the property losses recorded for these coatings after soil burial exposure are believed to be the result of chemical changes in these coatings. One of the least affected coatings in soil burial (N-12, polyimide) had no observable surface damage.

Several of the coatings (N-3, N-16, and N-17) had very rough surfaces which could harbor and partially protect contaminant microorganisms. A smoother surface would be preferable. Other coatings were very smooth at magnifications up to 10,000 X, but had pinholes, fissures, or changes in soil burial exposure. These were N-4, N-6, N-8, and N-10. Only N-12 (polyimide) had a smooth surface with no apparent loss in properties during soil burial. The remaining coatings (N-5 and N-14) examined had moderate smoothness with a moderate degree of property loss in soil burial.

Substances extracted from stainless steel (N-18) and untreated aluminum (N-20) stimulated the growth of most organisms evaluated in extract studies. Iridite-treated aluminum performed best in these evaluations by supporting less microbial growth. These findings were corroborated to some extent in petri plate and high humidity studies.

Of the four types of honeycomb materials evaluated, N-22 supported less microbial growth in the various studies than the other honeycombs, N-21, N-23, and N-24. Pit corrosion of the aluminum core material during microbiological exposures was abundantly evident in specimens N-21 and N-22. Because of the corrosion, the heat-resistant phenolic core in N-23 and N-24 seems preferable to the 5052-001 aluminum core used in the other specimens. The microbiological evaluations of these specimens cannot be fully interpreted since the history of handling before being received at Battelle was not known.

In conclusion, of seventeen externally-applied spacecraft coatings evaluated in extract, petri plate, high humidity, and soil burial experiments, none totally inhibited the test organisms employed and few were highly resistant to microbial deterioration under the conditions of the experiments conducted. The best of the group were N-2 (varnish), N-9 (phenolic-butyrate), and N-12 (polyimide). All should be improved by microbiocide addition or elimination of nutrient components in their formulations to minimize microbial growth or survival on coated spacecraft surfaces. By employing either approach, spacecraft sterilization procedures can be shortened with fewer adverse effects on heat-sensitive components of spacecraft systems. Metal substrates (stainless steel and aluminum) and honeycomb materials should be further investigated to determine more precisely their role in this situation.

The overall data are a good demonstration of the varied responses that can be obtained from vegetative and spore forms of the same organism; of the individual species responses that can occur due to the differences in physiology and make-up of the organisms investigated; and of the effect of water-soluble nutrients, stimulants, and inhibitors present in coatings and metal substrates. The data also indicate that a single experiment cannot be relied upon for judgements regarding the degree of microbial survival on or deterioration of materials.

RECOMMENDATIONS

We recommend that NASA consider the following approaches to improving externally-applied spacecraft coatings and related materials:

(1) Investigate suitable microbiocidal agents for incorporation into selected coatings.

(2) Encourage manufacturers to minimize water extractable nutrients in cured coatings.

(3) Encourage manufacturers to provide coatings with smooth surfaces.

(4) Investigate means of minimizing water extractable nutrients from metal substrates and honeycomb materials.

(5) Establish specifications for coatings, substrates, and honeycombs that require all of the above.