AVSSD-0318-67-CR

STERILIZATION ASSEMBLY DEVELOPMENT LABORATORY

"A Study of Chemical Residues
Left on Surfaces After
Biological Assay Procedures"

15 August 1967
TASK 6A

J.P.L. Contract #951624 - Phase II

THIS WORK WAS PERFORMED FOR THE JET PROPULSION LABORATORY, CALIFORNIA INSTITUTE OF TECHNOLOGY, SPONSORED BY THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION UNDER CONTRACT NAS7-100.

GPO PRICE $ _____

CFSTI PRICE(S) $ _____

Hard copy (HC) 3.00

Microfiche (MF) .65

ff 653 July 65

Prepared by

AVCO CORPORATION
SPACE SYSTEMS DIVISION
Lowell, Massachusetts

for

RECEIVED
NASA STI FACILITY
INPUT BRANCH
MAY 1968

JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

FACILITY FORM 602

N68-22520 (ACCESSION NUMBER)

(THRU)

20 (PAGES)

1 (CODE)

CR #94372 (NASA CR OR TMX OR AD NUMBER)

04 (CATEGORY)

-NOTE-

This report contains information prepared by AVCO Corporation, Space Systems Division, under JPL subcontract. Its content is not necessarily endorsed by the Jet Propulsion Laboratory, California Institute of Technology, or the National Aeronautics and Space Administration

STERILIZATION ASSEMBLY DEVELOPMENT LABORATORY

"A Study of Chemical Residues
Left on Surfaces After
Biological Assay Procedures"

15 August 1967

TASK 6A

J.P.L. Contract #951624 - Phase II

PREPARED BY: W. Gillchriest

W. Gillchriest
AVCO CORPORATION

APPROVED BY: E. A. Botan

E. A. Botan, Mgr. Microbiology
AVCO CORPORATION

APPROVED BY: E. J. Lunney

E. J. Lunney, C.S.O. Manager
AVCO CORPORATION

APPROVED BY: W. Paik

W. Paik, Sterilization
JET PROPULSION LABORATORY

APPROVED BY: G. H. Redmann

G. H. Redmann, SADL Project Mgr.
JET PROPULSION LABORATORY

JET PROPULSION LABORATORY

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA

A B S T R A C T

The object of this task was to evaluate the chemical residue left on the CMTM hardware after bioassay. Peptone deposits were found from the three liquid methods, swabbing, rinsing and washing. The washing method also left the detergent "Tween 80" detected by ultraviolet radiation fluorescence when combined with phosphine 3R.

The two solid methods, lift-off tape and rodacing, left residuals on the hardware which were removable with organic solvents. The rodacing method did not leave residual chloride.

The peptone residue was considered the least desirable from a biological burden point of view. "Tween 80" is considered less of a biological burden problem than peptone.

TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	EXPERIMENTAL PLAN	2
III.	MATERIALS AND METHODS	2
IV.	RESULTS	7
V.	DISCUSSION	10
VI.	CONCLUSIONS	12
VII.	RECOMMENDATIONS	13
VIII.	REFERENCES	14

I. INTRODUCTION

To determine what effects bio-assay procedures might have on spacecraft hardware materials, a study was made of the residuals left by the assay process. The determination of chemical residuals was performed by a combination of chemical and physical techniques. A brief theoretical evaluation was made of the possible effects produced by the residuals when found present.

The five methods of bio-assaying surfaces examined were:

1. Cotton Swabbing
2. Rodacing
3. Washing
4. Rinsing
5. Lift-off Tape

The methods used to determine residual deposits were: (1) Peptone-filter paper transfer technique, strips of Whatman #3 filter paper were moistened with water, pressed against the assayed surface removed, and then sprayed with ninhydrin solution[1]. An alternate choice was the direct spraying of the CMTM, or equivalent surface with ninhydrin. The ninhydrin examination was strictly qualitative for amino acids, short chain polypeptides and peptones. (2) Residual water was determined by the use of the Karl Fischer reagent[2]. This method also was only qualitative due to the differences in materials and their

ability to sequester free water. (3) Chlorides were "titrated" by the use of silver nitrate $(AgNO_3)$[3] aqueous solution. (4) Agar was detected by UV activation of a fluorescent indicator, ethidium bromide (EB), which was incorporated into the agar. (5) Detergent, "Tween 80" was determind by phosphine 3R[4] (a dye) which complexes with the fatty acid portion of "Tween 80".

II. EXPERIMENTAL PLANS

Surfaces of the CMTM or equivalent aluminum alloy, stainless steel alloy and plastic surfaces were treated with the Bio-assay techniques described in the int duction. Some subassemblies that contained the three materials to be tested were (1) the Rocket Motor (covered with plastic), (2) the Aeroshell (aluminum alloy), (3) the Bay Mounts (stainless steel alloy), and (4) the Impact Limiter (plastic covered).

A study was made to qualitatively determine the composition of residues deposited on CMTM or equivalent surfaces by surface Bio-assay methods. Only surface chemical contamination due to assaying techniques was investigated.

III. MATERIALS AND METHODS

A. The surfaces selected for examination were: (1) the Aeroshell, (aluminum surface), (2) the plastic-coated (balsa wood ball) Impact Limiter, (3) the Rocket Motor (fiberglass), (4) the Marmon Clamp (stainless steel), (5) the Bay Edges (stainless

steel), and (6) the Upperbay Canister (aluminum surface). Each area selected was divided into control and test area. No area was less than 2 in.^{2}, since these tests were qualitative, sample area would have minimal effect on the results. The selected areas were then cleaned: first with an Alconox solution, rinsed with tap water (3) times, then rinsed (3) times with distilled water, then with isopropyl alcohol and finally with diethyl ether if so required.

B. Swab Test

The assay area was designated "T" for test and "C" for control. UV assay (for cotton fibers), Karl Fischer Assay (for water), and the ninhydrin reaction (for peptone) were performed.

1. The surfaces assayed were swabbed according to the Preliminary NASA Standard Procedures for the Microbiological Evaluation of Spacecraft Hardware[5].

2. The swabbed areas were air-dried.

3. The surfaces were then wiped with a clean flannel cloth, moistened with ethanol.

4. Peptone Residual Determination

Strips of filter paper moistened with distilled water were applied to the swabbed surfaces. The filter paper was then removed, sprayed with ninhydrin and heated in a drying oven "to develop" the color of the ninhydrin-amino acid complex. The presence of peptone was indicated by a violet-colored spot on the filter paper.

Note: The ninhydrin test can be performed by directly spraying the surface with ninhydrin, thus avoiding the use of filter paper transfer (if practical).

5. Residual Water Determinations

Karl Fischer reagent was applied to the swabbed surface after it was air-dried. The Karl Fischer reagent will change from a brownish-black color to colorless, if water is present.

6. Cotton residue determinations.

The assayed areas were surveyed with a black light (UV source). The presence of residual cotton fibers would be shown by an intense blue-white fluorescence.

C. Rinsing Test

Each appropriately marked area was assayed for peptone, (ninhydrin) and residual water (Karl Fischer Reagent).

1. The area to be assayed was rinsed with 1% sterile peptone water, allowed to air-dry, and then wiped with a flannel cloth moistened with ethanol.

   a. The tests for residual peptone were performed as described in Sections C, 4 and 5.

D. Washing Test

Each appropriately marked area was assayed with the Karl Fischer reagent for water, with Phosphine 3R to determine the presence of "Tween 80" and with ninhydrin to determine the presence of peptone residual.

1. The surfaces assayed were washed with peptone water plus "Tween 80" detergent (0.1%).

2. The surfaces were allowed to air-dry.

3. Assays for residual peptone and water were carried out as described in Sections C, 4 and 5.

4. An ethanolic tincture 0.1% of Phosphine 3R was prepared.

5. The tincture was applied to the surface, allowed to remain on for 30 seconds to 1 minute and then blotted dry with Whatman #3 filter paper.

6. The surface was then examined with a UV light for fluorescence (light-green). If a "Tween 80" residue was present, fluorescence was observed.

E. Rodacing Test Areas

Each appropriately marked area was analyzed under UV light to detect the presence of ethidium-agar complex, which is indicative of an agar residue on the surface. Rodac tested areas were also assayed with a 0.1M silver nitrate solution for the detection of the chloride ion (Cl^-), with the Karl Fischer reagent for residual water and with the ninhydrin test for peptone.

1. After the surface was bio-assayed with the rodac plate, it was wiped down with a flannel cloth moistened with ethanol, and examined for the following residuals:

   a. Agar - a UV light was used to determine if residual agar was present. The residual ethidium-agar complex will give a red fluorescence when excited with UV.

   b. Chloride ion - the moistened filter paper technique as described in section C was used except that ninhydrin spray was replaced by a 0.1M $AgNO_3$ spray.

      (1) If chloride was present, a black spot of AgCl appeared.

      (2) If no black precipitate of AgCl was noted, the test area was irradiated with ultraviolet light as a further check. Ultraviolet light causes the degradation of AgCl to elemental Ag^o, which yields a black deposit.

F. Lift-off Tape Test Areas

Each appropriately marked and cleaned area was analyzed for the adhesive material by testing for an ethidium-adhesive complex residue. The tape was moistened with a 1% aqueous solution of ethidium bromide prior to use and allowed to drain free of the excess moisture before use.

1. The lift-off tape was applied to the surface to be assayed and then removed.

2. The assayed surface was then examined with an ultraviolet light for ethidium fluorescence (red).

G. Double Surface Masking Tape

A double surface masking tape which will be used to apply bio-assay coupons to CMTM surfaces was also evaluated using the same technique as in "F". The tape was applied to stainless steel and aluminum surfaces and was dry sterilized at 125°C. for 24 hours, to stimulate terminal sterilization.

1. The tape was removed and the surfaces evaluated for residual adhesive via the fluorescence technique.

IV. RESULTS (See Figure 1 for compilation of Data on Data Recording Form.)

A. Sensitivity Levels

FIGURE 1 - DATA RECORDING FORM

1. SWAB TECHNIQUE (air-dried)

	COTTON	WATER	PEPTONE
a. UV	(-)		
b. KARL FISCHER		Colorless(-) Black	
c. NINHYDRIN			Color(+) Colorless

2. RINSING TECHNIQUE (air-dried)

	PEPTONE	WATER
a. NINHYDRIN	(+)	
b. KARL FISCHER		(-)

3. WASHING TECHNIQUE (air-dried)

	WATER	PEPTONE	DETERGENT
a. KARL FISCHER	(-)		
b. NINHYDRIN		(+)	
c. UV			PHOSPHINE 3R Fluor

4. RODACING TECHNIQUES (air-dried)

	AGAR	HALOGEN	WATER
a. UV	Red(+) White		
b. $AgNO_3$		Black (-)White	
c. KARL FISCHER			(-)

5. LIFT-OFF TAPE TECHNIQUE (air-dried)

	ADHESIVE	WATER
a. UV	Red(+) White	
b. KARL FISCHER		(-)

1. The ninhydrin test for peptone as applied in this study could detect approximately 2.5×10^{-7} gms of peptone.

2. The silver nitrate test for chloride ions as applied in this study could detect approximately 1×10^{-7} gms of chloride ion.

3. The phosphine 3R test for "Tween 80" as applied in this study could detect approximately 1×10^{-7} gms of "Tween 80".

4. The ethidium bromide test for agar as applied in this study could detect approximately 10^{-9} gms. of agar.

5. The Karl Fischer test for residual water as applied in this study could detect approximately 10^{-12} gms. of water.

6. The ultraviolet test for detection of cotton fiber as applied in this study could detect cotton fibers which were barely visible to the naked eye, or visible to the limit of visual acuity.

B. Swabbing Tests

1. All surfaces assayed for peptone gave a positive reaction for peptone.

2. All surfaces assayed for cotton fibers were negative.

3. All surfaces assayed for residual water, when compared to the controls, were considered negative.

C. Rinsing Tests

1. All surfaces assayed for peptone gave a positive reaction for peptone.

2. All surfaces assayed for residual water, when compared to the controls, were considered negative.

D. Washing Tests

1. All surfaces assayed for peptone gave a positive reaction for peptone.

2. All surfaces assayed for residual water when compared to the control were considered negative.

3. All surfaces assayed for "Tween 80" (sorbitan monooleate) were positive for "Tween 80".

E. Lift-off Tapes and Double Back Masking Tape Tests

1. All surfaces assayed for the adhesives of lift-off tapes and double back masking tape were positive.

2. Approximately 75% or more of the adhesive residual could be removed when the surface was vigorously wiped with a flannel cloth moistened with ethanol.

3. When vigorously wiping the assayed surface with a flannel

cloth moistened with diethyl ether or acetone, approximately 95% or more of the residual adhesive is removed.

F. Rodac Tests

1. All surfaces assayed for peptone yielded a positive peptone reaction.

2. All surfaces assayed for residual water were considered negative when compared to the control.

3. All surfaces assayed for free chloride ion were considered negative when compared to the control. (See Discussion Section)

4. All surfaces assayed for residual agar yields a positive agar reaction.

V. DISCUSSION

All the bio-assay procedures evaluated in this study appear to leave a residual of assay material on the surface assayed. Some of these residuals could produce problems if they were left on flight hardware.

Peptones, for example, left by rinsing, washing, rodacing or swabbing could present a corrosion and/or microbial control problem. For example, the amino acids of the peptone could complex with anions or cations and produce corrosion on aluminum or aluminum alloy surfaces. Likewise, the amino acids of peptone can support microbial

growths which could present a biological burden control problem. In addition, the growth of microorganisms could produce organic acids and amines that could attack aluminum, aluminum alloys, plastics and possibly stainless steel surfaces.

Residual agar left by rodacing could present two problems. The first could be due to the dissolved peptone in agar and the second due to the agar itself. The agar residue could act as a "fly trap" for bacteria and thus present a problem of increased biological burden, while the peptone could present a corrosion and biological burden problems.

The "Tween 80" residue left by the washing procedure could produce a biological burden control problem and a possible corrosion problem. "Tween 80" is a sorbitan monooleate and is capable of supporting microbial growth.

An unexpected result of this study was the fact that free chloride ion was not detected in this study after the rodacing procedure. The Trypticase Soy Agar in the Rodac plate contained 5 gms of NaCl or 3.04 gms of chloride ion per liter of agar medium assuming 100% ionization. It was further assumed that approximately 0.1 ml to 0.01 ml of agar and associated fluid was dried on the assayed surface. Therefore, there could have been 0.30 to 0.03 mg. of Cl^- present on the surface after assay. This level is well within the sensitivity range

for chloride ion test conducted in this study. It is therefore postulated that the chloride ion might not have been present as a free ion, but complexed to some compound. Good candidates for the complexing compounds could be the peptone in the medium or other by-products present in the Trypticase Soy Agar. It would be of value to investigate the chemical state of the chloride ion for chloride ion is a well-known factor in producing corrosion.

As for the residual adhesives left by the lift-off tape and double surface masking tape, it is felt that they present no insoluble problem. The residual adhesives can be partly removed with ethanol and almost entirely with acetone or diethyl ether.

VI. CONCLUSIONS

1. The Bio-assay procedures examined leave chemical residuals on the surfaces assayed.

2. Peptone is considered the residual that could most seriously hamper control of biological burden.

3. Residual "Tween 80" is considered a lesser problem than residual peptone from a burden control viewpoint.

4. Residual peptone and "Tween 80", when acted upon by microorganisms could be factors involved in corrosion production.

VII. RECOMMENDATIONS

1. Modify or develop bio-assay procedures so that chemical residues, which could present biological burden control and/or corrosion problems are not left on surfaces after assays.

2. Investigate the chemical state of the chloride ion deposited on surfaces during the rodac plate assay, due to its potential role in corrosion production.

VIII. REFERENCES

1. West, E., et al., Textbook of Biochemistry, 4th edition, MacMillan Co., New York City, 1966.

2. Mitchell, J., Aquametry, Interscience Publishers, New York City, 1966.

3. Gomori, G., Microscopic Histochemistry, University of Chicago Press, 1952

4. Pearse, A., Histochemistry, Little, Brown and Co., Boston, 1960.

5. NASA Headquarters, Preliminary NASA Standard Procedures For The Microbiological Evaluation of Spacecraft Hardware, Washington D.C., 1966.