

M. Liverston

AMRL-TR-74-144

ADA 011539

citation



MICROBIOLOGICAL EVALUATION OF AVIATION FUEL STORAGE, DISPENSING AND AIRCRAFT SYSTEMS

SHELDON A. LONDON, PhD
AEROSPACE MEDICAL RESEARCH LABORATORY

NOVEMBER 1974

Approved for public release; distribution unlimited.

20060712054

STINFO COPY

**DIRECTORATE OF AEROSPACE FUELS
SAN ANTONIO AIR LOGISTICS CENTER
AIR FORCE LOGISTICS COMMAND
KELLY AIR FORCE BASE, TEXAS**

**AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433**

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Organizations and individuals receiving announcements or reports via the Aerospace Medical Research Laboratory automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address or cancellation.

Do not return this copy. Retain or destroy.

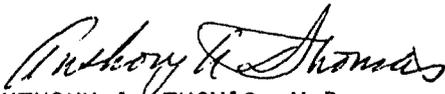
Please do not request copies of this report from Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22151

This report has been reviewed and cleared for open publication and/or public release by the appropriate Office of Information (OI) in accordance with AFR 190-17 and DODD 5230.0. There is no objection to unlimited distribution of this report to the public at large, or by DDC to the National Technical Information Service (NTIS).

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ANTHONY A. THOMAS, M.D.
Director, Toxic Hazards Division
6570th Aerospace Medical Research Laboratory

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AMRL-TR-74-144	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) MICROBIOLOGICAL EVALUATION OF AVIATION FUEL STORAGE, DISPENSING AND AIRCRAFT SYSTEMS		5. TYPE OF REPORT & PERIOD COVERED Final Report - 1 June to 31 August 1974
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Sheldon A. London, PhD		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Aerospace Medical Research Laboratory Aerospace Medical Div, Air Force Systems Command Wright-Patterson Air Force Base, OH 45433		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F; 021ATC01
11. CONTROLLING OFFICE NAME AND ADDRESS Aerospace Medical Research Laboratory Aerospace Medical Div, Air Force Systems Command Wright-Patterson Air Force Base, OH 45433		12. REPORT DATE November 1974
		13. NUMBER OF PAGES iii + 19
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Microbiological Survey Fuel System Icing Inhibitor Jet Fuel Aircraft AvGas Fuel Distribution System Water		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Recent unconfirmed reports of problems in refueling systems induced by microbial activity prompted an investigation of refueling facilities at four Air Force bases. The installations, selected as being representative of different operational bases and most likely to exhibit significant microbial contamination, were Homestead AFB, FL; Tyndall AFB, FL; Charleston AFB, SC; and Howard AFB, CZ, Panama. The distribution and servicing systems and aircraft surveyed were remarkably free of water, only 26 of 66 attempts to obtain water being successful. Those samples in which water was found contained considerably less than		

Block 20 (Continued)

observed in previous field investigations. Fuel System Icing Inhibitor, known to exert an inhibitory effect on microorganisms, was present in the water bottom samples in concentrations ranging from 7 to 25% by volume. Microbial levels, both fungal and bacterial were inconsequential. This survey has shown the facilities evaluated to be free of significant microbial contamination, attesting to the beneficial results of excellent housekeeping procedures. Assuming these results are indicative of Air Force systems in general, the status has not changed since the last survey conducted in 1965.

PREFACE

The microbiological survey described in this report was conducted during June and July 1974 by Dr. S. A. London, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson AFB, and Major R. A. Mathison and F. P. Morse, Directorate of Aerospace Fuels, San Antonio ALC, Kelly AFB. This study was initiated by the Air Force POL Technical Assistance Team under the provisions of AFR 74-7.

The author is indebted to Major Mathison and Mr. Morse for the coordination of the entire field survey, the excellent logistic support and the FSII analyses. The on-site support provided by fuel management and quality control personnel is greatly appreciated. The author would also like to express his sincere thanks to Ms. L. Jeannine Petri for her excellent technical assistance, and to Major R. Madson for the fungal identification.

INTRODUCTION

The growth of microorganisms in hydrocarbon environments is a well-documented phenomenon and has resulted in a specialized field of microbiological research (Beerstecher, 1954; Davis, 1967; Sharpley, 1961; Sharpley, 1964; Zobell, 1946). The knowledge accumulated over the past five decades has indicated the ubiquitous presence of a variety of microbial types in different hydrocarbon environments. Individual studies have been concerned with the species of microbes surviving and metabolizing in the presence of hydrocarbons, the rates of assimilation/degradation, the preference for particular kinds of hydrocarbon molecules, the influence of physical and chemical factors in the environment, microbial inhibition by hydrocarbons, and deleterious effects on hydrocarbon-containing environments induced by microbial activity. The latter area of research has been prompted by pragmatic needs to rectify destructive effects occurring in certain operations of the petroleum industry. Suffice it to say, in any reasonably moderate temperature environment that is comprised in part of hydrocarbons, microbial activity will occur (Cundell and Traxler, 1973; Ahearn, Meyers and Standard, 1970).

A recent interest in hydrocarbon microbiology was initiated by environmental pollution concerns and the potential application of petroleum degrading microorganisms as a means of control or abatement for oil spills (Nadeau and Roush, 1972). Another area receiving considerable effort is the formation of useful materials, particularly edible protein, through the metabolic transformation of hydrocarbons by specific microbes (Davies, Zajic and Wellman, 1973).

In light of the well-documented presence of microorganisms in petroleum products, the observation of microbial contamination in aircraft refueling systems was not surprising, at least not to microbiologists. As described by several investigators (Bakanauskus, 1958; Finefrock and London, 1966; London, Finefrock, and Killian, 1964; Prince, 1960) bacteria, yeasts and fungi were isolated from all segments of U. S. Air Force fuel distribution systems - from bulk storage tanks to aircraft integral wing tanks. The presence of these microbes, often in very high numbers and essentially found only in the ever-present water bottoms, was considered sufficient evidence that these entities were often the primary cause of fuel system problems, including sludge formation and subsequent filter clogging, fuel degradation, capacitance gauge malfunction, degradation of aluminum sealants and top coatings, and corrosion of aluminum alloy skins in so-called wet-wing aircraft. Considerable research conducted in the laboratory in conjunction with numerous field evaluations resulted in certain conflicts of opinion regarding the primary nature of microbial involvement; however, all researchers and fuel system engineers agreed that water was an indispensable factor in fuel system problems. More recently, the introduction of reticulated polyurethane foams in integral wing fuel-tanks as a means of retarding fuel surging and suppressing fire or explosion raised the possibility of increased microbial contamination due to the significantly increased surface area which is conducive to microbial growth. Several studies indicated bacterial and/or fungal activity may result in decreased tensile strength of the foams (Cooney and Felix, 1969; Edmonds and Cooney, 1968; Hedrick, 1969). Again, the significance of the presence of water was shown in another study of foam susceptibility to microorganism attack (Rogers and Kaplan, 1970).

In 1962 the Air Force added the compound ethylene glycol monomethyl ether to JP-4 to prevent ice formation in aircraft. In a short time this substance was observed in water bottoms, obtained from various locations throughout the distribution system, in concentrations as high as 40% volume. Laboratory studies indicated that the anti-icing compound, now termed Fuel System Icing Inhibitor (FSII), exerted microbiocidal effects. The presence of FSII in conjunction with concerted efforts by fuel handling personnel to eliminate the presence of water in ground systems ultimately resulted in the reduction of microbial contamination to inconsequential levels and the disappearance of fuel system problems attributable to microorganism activity. This elimination of microbiologically mediated malfunctions, corrosion, etc., resulted in the cessation of further laboratory and field studies in 1965.

Recently unconfirmed reports of possible deleterious microbial activity in commercial and military refueling systems prompted action by the Air Force POL Technical Assistance Team to perform a field survey of representative Air Force installations. On approval of the parent command, four locations were chosen on the basis of their operational activity and environmental conditions conducive to bacterial and fungal growth. Bases selected for evaluation were Homestead AFB, Florida; Tyndall AFB, Florida; Charleston AFB, South Carolina; and Howard AFB, Canal Zone, Panama. The purpose of this report is to present the results of this field survey and evaluation. While the evaluation was primarily directed to microbial levels in JP-4 systems, a few samples were analyzed from 115/145 and JP-5 systems.

MATERIALS AND METHODS

Procedures for the isolation, enumeration and identification of bacterial and fungal contaminants of aircraft fuels (present only in the associated water bottoms) have been previously established for laboratory studies and modified for application in the field. The later approaches did not suffer from restrictions imposed by logistic considerations since these extensive efforts warranted the use of military aircraft that enabled transport of essentially complete laboratory requirements. The present investigation was construed as a rather cursory survey and was complicated by the need to evaluate four different Air Force fuel distribution systems. This necessitated a redesign of field methods in such a fashion that all media and ancillary equipment could be carried in one case. The factors that were considered in selection of methods and associated equipment were:

1. Isolation of anaerobes.
2. Detection of sulfate reducing organisms (Desulfovibrio desulfuricans).
3. Isolation and identification of fungi.
4. Enumeration of aerobic bacteria.
5. Accurate measurement of analyzed samples.
6. Presterilized media in individual, leakproof containers.
7. Methods for sterilization of sampling points and devices.
8. Sufficient equipment to conduct analyses of 40 water-bottom samples.

Since space was a significantly limiting factor, arrangements were made with each Fuels Management Officer (FMO) to provide sterile glass, 4 ounce

specimen bottles available from the local Air Force dispensary. Additionally, sampling probes, Bacon bomb samplers and pump samplers were also provided by the FMO. These were sterilized in the field by rinsing with a 1:1000 aqueous dilution of benzalkonium chloride (Zephiran[®]) and subsequent rinsing with dry fuel. Sampling points on aircraft, filter separator units, refueling units, etc., were also sterilized with Zephiran.

The addition of water-bottom samples to the various media was accomplished quantitatively with sterile disposable glass tuberculin syringes fitted with sterile 18 gauge, disposable, 1.5 inch needles.

For the detection of anaerobic bacteria, 9.0 ml of Fluid Thioglycollate Medium (Difco) in a 10 ml screw-capped lyophilization vial (Virtis 10 - 159) was inoculated through the split rubber stopper with 0.5 ml of water-bottom sample.

The presence of sulfate reducers was determined by inoculation of 1.0 ml of water-bottom sample into 9.0 ml of commercially prepared and bottled Sulfate API Broth (Difco #0500-86). Fungi were isolated by spreading 0.1 ml on Cooke Rose Bengal Agar (Difco) containing 35 mg/ml Chloromycetin[®]. This medium was prepared in the laboratory and added aseptically to plastic, disposable Petri dishes (Millipore[®] PD10 047 00). Media used subsequently in the laboratory for fungal identification included Corn Meal Agar (Difco), malt extract agar, and Cooke Rose Bengal Agar. Identification was based upon morphological criteria according to Barnett (1962). Quantitative determination of aerobic bacteria was accomplished by filtering 1.0 ml of sample in a Millipore Field Monitor (No. MHWG037P0). Negative pressure for filtration was available at the fuel quality control laboratory at each Air Force base. Double strength Trypticase Soy Broth (BBL) was prepared in 30 ml quantities in 50 ml serum stoppered vials and was added to the filter pad in the Field Monitor by

syringe (approximately 0.5 ml). The media were inoculated within two hours after the samples were obtained and incubated at ambient temperature (which varied from approximately 21°C to 34°C). Observation of results occurred in the laboratory at Wright-Patterson AFB. Since samples were obtained sequentially, incubation times varied from three to four weeks.

Concentrations of FSII in the water bottoms were determined with a refractometer. Water-bottom samples were obtained as follows: aircraft - drain plugs; bulk storage tanks - drain valve; operating tanks - Bacon bomb or pump sampler; all others - drain valve.

RESULTS AND DISCUSSION

Prior to initiating the survey, we requested each FMO to refrain from draining fuel servicing equipment for several days before sampling would be performed. This was done in an attempt to insure that water-bottom samples would be available. Special efforts were not taken to advise aircraft maintenance personnel on draining of aircraft. A minimum of ten samples per base was considered adequate for a representative indication of fuel system contamination based upon previous experience and logistic restrictions. A total of 66 attempts to obtain water was made. Table I lists the 26 samples of water bottoms that were available while Table II indicates the 40 sources in which water could not be found. Many of the samples consisted of only a few milliliters, e.g., sample No. 9 represents a pooling of water obtained from two Firebee drones in order to provide a sufficient quantity for analysis. With the exception of some floating roof bulk tanks, copious amounts (greater than 100 ml) of water were not found. During the early field surveys mentioned previously, quantities of water in excess of four liters were often drained from points throughout the fuel system and aircraft. The gross reduction of free water at the bases evaluated attests to the excellence of fuel management and aircraft servicing personnel in adhering to operating and quality control procedures.

On the basis of this very low level of water in conjunction with concentrations of FSII ranging from seven to 25% by volume, as shown in Table VII (samples five and 23 were obtained from AvGas systems which do not contain FSII), one would expect a very small amount of microbial contamination. This was borne out by the microbiological analyses, the results of which are presented in Tables III, IV, and V.

Significant growth occurred from 12 (46.2%) of the samples in Thioglycollate and 10 (38.5%) in Sulfate API (Table III). Gram stains revealed pure cultures in most cases with gram positive cocci predominating. This is somewhat in contradiction to earlier findings in that the facultative anaerobe Pseudomonas sp. (a gram negative rod) was isolated most frequently. The presence of gram negative cocci is also of interest in that these organisms are usually associated with animals and not generally observed in the soil or as airborne forms. These gram negative cocci were not isolated from Sulfate API. None of the organisms capable of growth in Sulfate API could effect the reduction of sulfate. Comparison of the results from Thioglycollate with Sulfate API shows almost no correlation. This indicates that the environments provided by these two media were conducive to the growth of different bacterial types. Table IV lists those instances in which bacteria were isolated on CRB agar. This is of interest since bacterial growth should be inhibited on this medium due to the incorporation of an antibiotic. The sporadic appearance of bacteria on this medium cannot be correlated with growth occurring in the anaerobic media, again indicating that different media enable the isolation of various microbial types. This emphasizes the necessity to utilize various microbiological procedures to insure that the divergent microbial types present are recovered.

The results obtained with the Millipore Field Monitor (for the enumeration of aerobes), shown in Table III, were not anticipated. The only significant growth occurred from samples five and 23; both are water-bottoms from AvGas systems. The three colonies observed from sample 11 do not correlate with the other analyses of this sample and therefore may be due to experimental contamination. The lack of growth from all other samples can be attributed to one of the following: 1) the samples contained less than one aerobic colony-forming

unit/ml; 2) the viable cells present were not capable of growth in TS broth; or 3) since the filters were not rinsed with a surfactant such as Triton[®] X-100 subsequent to sample filtration, the TS broth in the filter pad could not diffuse through the filter and provide a growth substrate. In earlier studies the washing of the filter with a sterile solution of surfactant was considered a routine procedure to clear the filter of entrained JP-4. For this study it was not utilized since the samples were allowed to settle completely prior to analysis and caution was exercised to insure only water was withdrawn in the syringe. The Field Monitors were checked in the laboratory, prior to performing the survey, with an aliquot of the TS broth used in the field and gave acceptable results. In addition, the presence of three colonies from sample 11 indicates that the procedure used was adequate to permit bacterial growth. Thus, the third possible explanation is rather unlikely. The second possibility does not appear feasible since TS broth is a widely used medium and has proven to be an excellent general growth environment for microbes present in JP-4 systems. Therefore, the lack of growth on the Field Monitors is probably attributable to a very low level of aerobic microbial contamination in the water-bottom samples examined.

Table IV presents an enumeration of the various fungal genera observed. Identification of fungal genera was accomplished to determine if microbial fuel system contaminants had changed since the previous investigations. Fungal rather than bacterial identification was selected since it is a rather rapid and easy procedure and information on previously observed genera was available. Since each CRB agar plate was inoculated with 0.1 ml, the maximum count obtained was 150 colony-forming units/ml from sample 22. Of the 26 samples examined, 15 (57.7%) exhibited the presence of fungi, and only four of these (Nos. 19-22)

showed more than three colonies. These samples, containing 120 to 150 colony-forming units/ml, were obtained from bulk storage tanks at Howard AFB. Table V is a compilation of total number of colonies observed (by genus) and their frequency of observation. These data are quite consistent with earlier findings that Cladosporium sp. (also designated Hormodendrum sp.) and Penicillium sp. were the most consistently observed fungal contaminants.

These microbiological analyses of the 26 water-bottom samples provide definitive information concerning the contaminant status of the four bases surveyed. The exceedingly low levels of viable microbial growth are totally inconsequential and essentially are in agreement with observations made previously. For comparative purposes, Table VI, taken from London et al., 1964 is presented to indicate the similarity of findings. Some of the early investigations of fuel system contamination reported concentrations of microorganisms as high as 10^8 /ml. The levels observed during the present survey and those presented in Table VI indicate the significant reduction of microbial contaminants that has been maintained for approximately 10 years. It is quite evident that improvements in fuel system design, maintenance, operations, and quality control coupled with the presence of FSII have eliminated the concern for microbial contamination and concomitant fuel system degradation. Periodic field surveys of bases likely to experience consequential microbial contamination should be initiated as an effective means to determine fuel system conditions and should be construed as another aspect of preventive maintenance.

Table 1. Description of Water-Bottom Samples

SAMPLE NUMBER	LOCATION	DATE	SOURCE
1	Homestead	6-25	JP-5, PH#1, OT#3
2	Homestead	6-25	JP-4, PH#1, OT#2
3	Homestead	6-25	JP-4, BST#330
4	Homestead	6-25	JP-4, BST#331
5	Homestead	6-25	AvGas 115/145, BST#314
6	Homestead	6-25	JP-4, A/C F4-E #68311 - main tank
7	Homestead	6-25	JP-4, RU R-5 #68 L 30 - main tank sump
8	Tyndall	6-28	JP-5, FD #70-1638
9	Tyndall	6-28	JP-5, Pooled from FD #69-6746 and #69-6739
10	Tyndall	6-28	JP-4, BST #6046
11	Tyndall	6-28	JP-4, BST #6047
12	Tyndall	6-28	JP-4, BST #6045
13	Charleston	7-2	JP-4, A/C C141 #60162 - main tank #2
14	Charleston	7-2	JP-4, A/C C141 #40630 - main tank #3
15	Charleston	7-2	JP-4, BST #1
16	Charleston	7-2	JP-4, BST #5
17	Charleston	7-2	JP-4, RU R-5 #67 L 1306
18	Charleston	7-2	JP-4, PH#3, RT#5
19	Howard	7-5	JP-4, BST, main base tank #1
20	Howard	7-5	JP-4, BST, main base tank #6
21	Howard	7-5	JP-4, BST #199, initial bleed from drain line
22	Howard	7-5	JP-4, BST #199, after flushing drain line
23	Howard	7-5	AvGas 115/145, RU R-2 #62 L 368
24	Howard	7-5	JP-4, HC MH2A #58W0012 - filter separator sump
25	Howard	7-5	JP-4, OT#2 - filter separator drain
26	Howard	7-5	JP-4, OT#1 - bomb sample from hatch

OT = operating tank	RU = refueling unit
PH = pump house	FD = Firebee drone, BQM-34A
BST = bulk storage tank	HC = hose cart
A/C = aircraft	

Table II. Description of Sources Sampled for Water Unsuccessfully

LOCATION	SOURCE
Homestead	JP-5, PH#1, OT#3 - filter separator drain
Homestead	JP-5, PH#1, OT#1 - pump sample from bottom
Homestead	JP-4, A/C F4-E #66314
Homestead	JP-4, A/C F4-E #67332
Tyndall	JP-4, Four (4) RU R-5
Tyndall	JP-4, Four (4) A/C F106
Tyndall	JP-4, Four (4) A/C F101
Tyndall	JP-5, Two (2) FD
Charleston	JP-4, BST
Charleston	JP-4, Two (2) A/C C141
Charleston	JP-4, RU R-5
Charleston	JP-4, HC MH2A
Charleston	JP-4, PH#1, OT#1, #3, #5
Howard	AvGas, 115/145 BST
Howard	JP-4, Four (4) A/C C130
Howard	JP-4, Four (4) RU R-5
Howard	JP-4, HC MH2A
Howard	JP-4, Four (4) A/C A7D
<hr/> Total = 40 sources showing no water	

See Table I for abbreviations

Table III. Summary of Bacterial Activity in Water Bottoms

Sample Number	Thioglycollate		Sulfate API			Aerobes/ml ¹
	Growth	Gram Stain	Growth	SO ₄ Red.	Gram Stain	
1	±	P cocci	-			0
2	-		+	-	P cocco-bacilli	0
3	±	P rods	-			0
4	-		-			0
5	+	P cocci	+	-	N rods	116, n rods
6	-		-			0
7	+	P cocci	+	-	N rods	0
8	+	P and N cocci	-			0
9	+	P cocci	-			0
10	-		+	-	P cocci	0
11	+	P cocci	-			3, n rods
12	+	P and N cocci	±	-	N rods	0
13	-		+	-	P cocci	0
14	-		-			0
15	+	P cocci	+	-	P cocci	0
16	-		-			0
17	±	P rods	+	-	P cocci	0
18	+	N rods	-			0
19	±	N cocci	-			0
20	+	N rods, yeast	-			0
21	-		-			0
22	-		-			0
23	+	V cocci	-			TNTC
24	+	P cocci	+	-	P cocci	0
25	+	P cocci	+	-	P cocci	0
26	-		+	-	P cocci	0

N = Gram negative
P = Gram positive
V = Gram variable

± = Very slight growth
TNTC = Too numerous to count

¹Based upon colonies appearing on Millipore Field Monitor

Table IV. Summary of Growth on Cooke Rose Bengal Agar

Sample Number	Observation
1	10 bacterial colonies
2	Approximately 100 bacterial colonies
3	Negative
4	Negative
5	Bacterial colonies not countable
6	Negative
7	Negative
8	Bacterial colonies not countable
9	1 <u>Blodgettia sp.</u> , 1 <u>Helminthosporium sp.</u>
10	1 bacterial colony
11	1 <u>Aspergillus sp.</u> , 1 <u>Cylindrocephalum</u>
12	Negative
13	1 <u>Geotrichum sp.</u> , 1 unidentified, 16 bacterial colonies
14	1 <u>Epicoccum sp.</u> , 1 unidentified
15	> 300 bacterial colonies
16	1 <u>Alternaria sp.</u>
17	1 <u>Epicoccum sp.</u>
18	1 <u>Cytosporella sp.</u>
19	8 <u>Penicillium sp.</u> , 3 <u>Cladosporium sp.</u> , 1 <u>Curvularia sp.</u> , 1 <u>Zygodessmus sp.</u> , several bacterial colonies
20	5 <u>Penicillium sp.</u> , 3 <u>Cladosporium sp.</u> , 2 <u>Curvularia sp.</u> , 1 <u>Amerosporium sp.</u> , 1 unidentified, several bacterial colonies
21	5 <u>Cladosporium sp.</u> , 3 <u>Fusarium sp.</u> , 2 <u>Nigrospora sp.</u> , 2 <u>Penicillium sp.</u> , 1 <u>Helminthosporium sp.</u> , 1 <u>Curvularia sp.</u> , several bacterial colonies
22	7 <u>Cladosporium sp.</u> , 5 <u>Trichocladium sp.</u> , 3 <u>Penicillium sp.</u> , several bacterial colonies
23	1 <u>Epicoccum sp.</u> , 1 <u>Penicillium sp.</u> , 1 unidentified, TNTC bacterial colonies
24	1 <u>Cladosporium sp.</u> , 1 <u>Penicillium sp.</u> , 1 <u>Trichocladium sp.</u>
25	1 <u>Cladosporium sp.</u> , 1 <u>Aspergillus sp.</u>
26	2 <u>Cladosporium sp.</u> , 1 unidentified

Numbers refer to colony count of identified fungi assumed to result from one spore or hyphal fragment. Bacterial colonies often obscured or merged and thus not countable.

Table V. Occurrence of Fungal Genera

Genus	Total Number of Colonies	Frequency of Isolation
Cladosporium	22	7
Penicillium	20	6
Curvularia	4	3
Epicoccum	3	3
Aspergillus	2	2
Helminthosporium	2	2
Trichocladium	6	2
Alternaria	1	1
Geotrichum	1	1
Amerosporium	1	1
Fusarium	3	1
Blodgettia	1	1
Cylindrocephalum	1	1
Zygodemus	1	1
Nigrospora	2	1
Cytosporella	1	1
Unidentified	5	5
Total	76	

Table VI. Resume of Data From Ramey Air Force Base Field Survey, February 1964

Source of Sample	Bacterial count/ml	Anaerobes	Fungi	EGME % vol
PH1 RT56, W	<10	No	No	19
PH1 RT57, W	7	Yes	No	20
PH1 RT58, W	3	Yes	No	20
PH1 RT59, W	<10	No	No	17
PH1 RT60, W	10	Yes	No	19
PH1 RT61, W	20	Yes	Yes	20
PH1 RT62, W	<10	No	No	17
PH1 RT63, W	10	Yes	No	19
PH2, RT64, W	10	No	No	23
PH2, RT65, W	<10	No	No	—
PH2 RT66, W	Spreader	Yes	Yes	20
PH2, RT67, W	<10	Yes	No	21
PH2, RT68, W	10	Yes	No	26
PH2, RT69, W	150	Yes	No	20
PH2, RT70, W	<10	Yes	No	23
PH2 RT71, W	<10	No	No	—
PH3, RT72, W	210	Yes	No	21
PH3, RT73, W	130	Yes	No	18
PH3 RT74, W	<10	Yes	No	20
PH3 RT75, W	147	Yes	No	—
PH3 RT76, W	127	Yes	No	19
PH3 RT77, W	90	Yes	Yes	—
PH3 RT78, W	<10	Yes	No	24
PH3 RT79, W	83	Yes	No	20
BST24, W	763	—	No	14
BST25, W	770	—	No	17
BST55, W	37	—	No	12
TF6 RT38, W	127	Yes	No	18
TF6 RT39, W	1190	Yes	No	13
TF6, RT40, W	157	Yes	No	14
TF6 RT41, W	2130	Yes	No	20
TF6, RT42, W	973	Yes	No	13
TF6 RT43, W	6670	Yes	No	—
TF6 RT44, W	550	Yes	No	16
TF6 RT45, W	3340	Yes	No	—
PH1 RT58, FS, F	0	No	No	—
PH1 RT59, FS, F	0	No	No	—
PH1 RT62, FS, F	0	No	No	—
PH1 RT63, FS, F	0	No	No	—
AC6475 MT3, W	<10	Yes	No	—
AC6475 MT4, W	0	—	No	—
AC1501 pooled MT, W	0	—	No	—
AC6474 MT1, W	0	—	No	—
AC6474 MT4, W	0	—	No	—
AC0237 pooled MT, W	0	No	No	—
AC1502 MT3, W	0	Yes	No	—
AC1502 MT2, W	0	No	No	—

PH = pump house
 RT = ready tank
 W = water sample
 — = no data
 BST = bulk storage tank
 TF = tank farm

FS = filter separator
 F = fuel sample
 AC = aircraft
 MT = main integral wing fuel tanks
 EGME = ethylene glycol monomethyl ether

Table VII. Concentration of FSII in Water-Bottom Samples

Sample Number	FSII % by Volume
1	25
2	18
3	10
4	16
5	0
6	12
7	7
8	22
9	20
10	17
11	10
12	15
13	17
14	16
15	8
16	17
17	20
18	19
19	18
20	18
21	21
22	21
23	0
24	13
25	13
26	15

REFERENCES

1. Ahearn, D. G., S. P. Meyers, and P. G. Standard, 1970. The role of yeasts in the decomposition of oils in marine environments. *Dev. Ind. Microbiol.*, 12:126-134.
2. Bakanauskus, S. 1958. Bacterial activity in JP-4 fuel. WADC Technical Report 58-32, Wright Air Development Center, Wright-Patterson AFB, Ohio.
3. Barnett, H. L. Illustrated Genera of Imperfect Fungi, 2nd Edition, 1962. Burgess Publishing Company, Minneapolis, MN.
4. Beerstecher, E., Jr. 1954. Petroleum Microbiology, Elsevier Press, Inc., Houston, TX.
5. Cooney, J. J. and J. A. Felix 1969. Polyurethane foams and foam additives in hydrocarbon fuel-water systems. *Dev. Indust. Microbiol.*, 11:210-224.
6. Cundell, A. M. and R. W. Traxler 1973. Hydrocarbon-degrading bacteria associated with Arctic oil seeps. *Dev. Ind. Microbiol.*, 15:250-255.
7. Davies, J. S., J. E. Zajic, and A. M. Wellman 1973. Fungal oxidation of gaseous alkanes. *Dev. Ind. Microbiol.*, 15:256-262.
8. Davis, J. B. 1967. Petroleum Microbiology, Elsevier Publishing Company, Amsterdam.
9. Edmonds, P. and J. J. Cooney 1968. Microbial growth in a fuel-water system containing polyesterurethane foam. *Appl. Microbiol.*, 16:426-427.
10. Finefrock, V. H. and S. A. London 1966. Microbial contamination of USAF JP-4 fuels. AFAPL-TR-66-91. Air Force Aero Propulsion Laboratory, Wright-Patterson AFB, Ohio.
11. Hedrick, H. G. 1969. Observations of microbial growth on polyurethane foam in aluminum alloy tanks. *Dev. Ind. Microbiol.*, 11:163-172.

12. London, S. A., V. H. Finefrock and L. N. Killian 1964. Microbial activity in Air Force jet fuel systems. *Dev. Ind. Microbiol.*, 6:61-79.
13. Nadeau, R. J. and T. H. Roush 1972. Biological effects of oil pollution. Selected bibliography II. EPA-R2-72-055, U. S. Environmental Protection Agency, Cincinnati, Ohio.
14. Prince, A. E. 1960. Microbiological sludge in jet aircraft fuel. *Dev. Ind. Microbiol.*, 2:197-203.
15. Rogers, M. R. and A. M. Kaplan 1970. Microbial susceptibility of reticulated polyester urethane foam in simulated fuel tank environments. *Dev. Ind. Microbiol.*, 12:393-403.
16. Sharpley, J. M. 1964. Elementary Hydrocarbon Microbiology, ASD-TDR-63-752 (AD 600-232), Aeronautical Systems Division, Wright-Patterson AFB, Ohio.
17. Sharpley, J. M. 1961. Applied Petroleum Microbiology, Buckman Laboratories, Inc., Memphis, TN.
18. Zobell, C. E. 1946. Action of microorganisms on hydrocarbons. *Bact. Rev.* 10:1-49.