# EVALUATION OF A BIOCIDAL TURBINE-FUEL ADDITIVE

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## I. Introduction.

Microbial growth in kerosene-type fuels is often associated with the fouling of fuel screens and capacitance probes, as well as the corrosion of aluminum-alloy fuel tanks (1–13). Since 1956, when fuel system malfunctions in the B–47 aircraft were traced to microbial sludge formation, considerable effort has been expended to develop a fuel additive which would retard the growth of these organisms and, at the same time, be compatible with the fuel system components.

Of particular interest recently has been the potential usefulness of several boron-containing organic compounds which exhibit microbiocidal properties[2,3,10,14,15]. These compounds present a unique solution to the problem of getting the biocidal material into contact with the organisms which proliferate in the water layer, since they could be introduced as a fuel-biocide solution and, due to their water solubility, would tend to saturate any water pockets which exist in the tank. Biobor-JF, developed by the Standard Oil Company of Ohio, is such a product.

An evaluation of the effectiveness of Biobor-JF as a biocidal jet fuel additive was initiated in a two-phase program. One phase was a carefully controlled laboratory study of the biocidal properties of the additive under simulated field conditions. Concurrent tests were conducted by the Aircraft Services Base, Federal Aviation Administration, Oklahoma City, Oklahoma using the fuel-additive mixture in an agency-owned Convair 880 aircraft performing routine flight operations. This report, however, is concerned primarily with the laboratory studies.

The laboratory studies conducted in this evaluation consisted of the following:

a. Comparative evaluation of seven nutrient media to determine the one most suitable for culturing the mixed population of microorganisms found in fuel.

b. Evaluation of a membrane filter procedure for measuring microbial concentration in both fuel and aqueous samples.

c. Viability of microorganisms in turbine fuel.

d. Kinetics of microbial mass transfer in fuel-water systems.

e. Effect of Biobor-JF on microbial growth in fuel-water systems.

f. Effect of Biobor-JF on microbial growth in liquid and solid nutrient media.

## II. Experimental.

*Fuel.* A commercial kerosene fuel, Conoco Jet–50, was used in all laboratory and field studies except as noted in Table IX.

*Counting of Microorganisms*

1. Fuel Samples: Microorganisms in jet fuel were counted using a modification of the procedure described in the Society for Industrial Microbiology publication, "Proposed Procedures for Microbiological Examination of Fuels"[16]. The measured fuel samples were filtered by suction through membrane filters (Millipore membrane, type HA, 47–mm dia., pore size $0.45\mu$) supported in sterile, stainless steel filter funnels (Gelman Instrument Co.) of approximately 1 liter capacity. Each filter was washed with approximately 300 ml of sterile 0.1% aqueous Triton X–100[1] to remove the residual kerosene from the membrane, followed by 100 ml rinse with 0.01 M phosphate buffer, pH 6.8. The membrane filter was transferred aseptically from the filter assembly to a petri dish (Lab-Tek Plastics Co., 60 x 20 mm) containing the appropriate growth medium. The filter was placed on the agar, face-up, covered, and incubated.

2. Water Samples: Microorganisms in water samples were serially diluted with 0.01 M phosphate buffer, pH 6.8, and filtered through membrane filters as described above except that they did not receive the detergent wash.

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[1] Triton X–100 is an alkyl phenoxy polyethoxy ethanol produced by Rohm and Haas Company, Philadelphia, Pennsylvania.

*Media Selection.* Media tested for use with the membrane filter counting method included Nutrient Agar (Fisher Scientific Co.), Standard Plate Count Medium (Baltimore Biological Laboratory), Double Strength Nutrient Agar, Tryptone-Glucose Extract Agar (Difco Manual #9, p. 57), Nutrient Broth enriched with 0.1% yeast extract and 0.1% glucose, Sabouraud Dextrose Agar (Fisher Scientific Co.) and Czapek Solution Agar (Difco Manual #9, p. 245). Replicate samples were withdrawn from a constantly-stirred suspension of mixed bacteria and fungi which had been isolated from fuel. Each sample was filtered and the filters incubated at 37°C on poured plates of the above test media. Counts of bacterial colonies present after 24, 48, and 72 hours of incubation on the first five media failed to show any significant differences among the various media in terms of the number of colonies or rate of growth. Of the last two media, which are designed primarily for the cultivation of fungi, Sabouraud Dextrose Agar supported fungal growth considerably better than the Czapek Solution Agar. Nutrient Agar and Sabouraud Dextrose Agar were therefore selected for the cultivation of bacteria and fungi respectively.

*Accuracy of Membrane Filter Method.* To establish the degree of precision to be expected from the membrane filter method of counting, the following experiments were conducted.

1. Aqueous Suspensions: Separate suspensions of bacteria and fungi were prepared in phosphate buffer (0.01 M, pH 6.8) by the direct addition of a diluted broth culture of the organism. Replicate samples were prepared by transferring 1.0 ml samples from each constantly-stirred microbial suspension to the sterile funnel-filter assembly which contained 100 ml of phosphate buffer. The addition of the 1.0 ml sample to the buffer provided a more uniform distribution of the organisms over the filter surface. After application of suction, an additional 100 ml of sterile buffer was used to wash down the sides of the funnel.

2. Fuel Suspensions: Separate suspensions of bacteria and fungi in fuel were prepared by shaking fuel with a broth culture of each organism, allowing the broth to settle, then carefully decanting the fuel. Samples of 100 and 500 ml volumes were removed from the constantly-stirred fuel suspension and transferred to the

filter-funnel assembly, then washed with detergent and buffer as previously described.

The washed filters from both aqueous and fuel suspensions were then transferred to petri dishes containing the appropriate medium and incubated at 34°C. Colony counts were made using a standard Quebec counter after 24 hours of incubation for the bacterial plates and 48 hours for the fungal plates. The results are shown in Table I; the coefficient of variation ranged from 5% to 12%.

*Detergent Effect.* Since the jet fuel must be removed from the membrane filter prior to incubation to allow the nutrient to diffuse to the entrapped cells, a detergent rinse is necessary. To determine the effect of this detergent on the growth of the bacteria, aliquots from suspensions of known species (both gram-positive and gram-negative) were transferred into approximately 10 ml of sterile, aqueous 0.1% Triton X–100 contained in a sterile filter-funnel unit. After removal of the detergent solution by suction, the filter was washed with 0, 25, 100, or 500 ml of sterile phosphate buffer (0.01M, pH 6.8); the 500 ml rinse was added in five 100-ml portions to assure complete removal of the detergent. Colony counts were made after 26 hours of incubation on nutrient agar at 34°C.

*Settling Rate.* In order to estimate the settling rate of bacteria placed in suspension by tank turbulence, a laboratory tank was prepared using a 2-liter, stoppered, graduated glass cylinder. Each such tank was inoculated with 100 ml of Bushnell-Haas salts solution[17] containing 2 ml of a suspension of 2 bacterial and 2 fungal types previously isolated from fuel. 1900 ml of fuel was layered over the 100 ml of salts solution. After 8 days' incubation at room temperature, the cotton plug was replaced by a glass stopper and the fuel-water mixture was shaken vigorously. At various time intervals after shaking, samples of 25 ml each were aseptically withdrawn from exactly 4 inches below the fuel surface, plated, and counted.

*Distribution of Organisms in Fuel-Water System.* To establish the distribution of bacteria in fuel tanks under static conditions, 3 "tanks" were prepared as described above and incubated for 8–12 days at room temperature. Using a long, sterile, tubular probe, fuel samples were removed at various distances above the fuel-water interface and from the water layer itself;

these samples were then filtered, plated, incubated, and counted.

*Long-Term Growth of Microorganisms in Fuel Phase.* To determine whether microorganisms can live and multiply in jet fuel without the presence of a discrete water phase, several gallons of untreated jet fuel were chilled in a cold room (3°C) for 3 days and filtered (Whatman #1 filter paper) into a dry container. This system contained such organisms as were indigenous to the fuel and only that amount of water which could be maintained in solution at 3°C; no separate, discrete water phase was present when the system was brought to room temperature. Samples were removed for counting immediately after filtration and at intervals up to 63 days. Incubation temperature was 23–25°C for this 63–day period.

*Inhibition of Microbial Growth in Fuel-Water Systems by Biobor-JF.* To study the rate and extent of microbial inhibition by Biobor-JF in a fuel-water system, four laboratory "tanks" were prepared using 2-liter graduated cylinders stoppered with cotton. 1950 ml of untreated commercial jet fuel were placed in each cylinder. Two bacterial and 2 fungal isolates were grown in separate broth cultures, and 10 ml of each culture were withdrawn, mixed together, and washed twice with Bushnell-Haas salts solution followed by centrifugation. The final mixed suspension was added to 1 liter of the sterile salts solution and a 50 ml aliquot of this suspension was added to each tank. Tanks 1 and 2 were designated as controls; tanks 3 and 4 were treated by adding Biobor-JF to the fuel layer to a concentration of 270 ppm by weight (equivalent to 20 ppm elemental boron). After initial mixing, samples were removed for counting at intervals up to 33 days. Two hours after a uniform agitation of 4 inversions (to simulate normal refueling turbulence), samples were taken with a sterile probe from the aqueous layer and from the fuel layer (4 inches below the fuel surface).

*Inhibition of Microbial Growth on Solid Nutrient Media by Biobor-JF.* Eight bacterial and 5 fungal isolates, selected from a total of 19 apparently discrete types isolated from fuel (see Appendix A)[1], were grown in separate broth cultures; these were uniformly inoculated onto the surfaces of individual petri dishes of solid media using a sterile glass rod spreader. Bacteria and fungi were grown on nutrient agar and Sabouraud Dextrose Agar respectively, using a single isolate per dish. Whatman #2 filter paper was cut into 6-mm discs using a paper punch; the sterilized discs were dipped into Biobor-JF and applied to the surface of the inoculated plates. Plates were inspected for a zone of inhibition after a 48-hour incubation at 34°C.

In a second experiment, Biobor-JF was added directly to nutrient agar and Sabouraud Dextrose Agar to a final concentration of 400 ppm; it was added after steam sterilization but before the agar had solidified. Petri dishes were poured with the treated agars and inoculated as previously described.

A third experiment was designed to determine whether the microorganisms could utilize Biobor-JF as a sole source of carbon. After sterilization of a solid medium prepared by adding 15 g/liter of agar to Bushnell-Haas salts solution, Biobor-JF was added in concentrations of 0, 4,000, and 10,000 ppm. Four different bacterial isolates were inoculated onto each of the 3 media.

*Inhibition of Microbial Growth in Liquid Nutrient Media by Biobor-JF.* Flasks containing 5 ml of sterile nutrient broth (beef extract 0.3%, peptone 0.5%), in which 0, 200, 500, 1,000, 5,000, or 10,000 ppm of Biobor-JF was incorporated, were separately inoculated with 1-drop suspensions of broth cultures of individual bacterial isolates. These flasks were cotton-stoppered and incubated with constant shaking at room temperature (26°C) for 22 hours. Optical density of the bacterial suspension was determined at 600 m$\mu$ using a Coleman Junior spectrophotometer with nutrient broth as the blank.

For fungal inhibition studies, separate flasks containing 75 ml of sterile Sabouraud Dextrose broth in which 0, 200, 500, 1,000, 5,000, or 10,000 ppm of Biobor-JF was incorporated (after sterilization) were inoculated with 1-drop suspensions of broth cultures of single fungal isolates from jet fuel. The flasks were incubated at room temperature (26°C) for 5 days with intermittent shaking. The medium was removed by filtering the entire flask contents through a membrane filter (Millipore, Type RA, pore dia. 1.20$\mu$, filter diameter 47 mm); the mycelium was then washed with 20 ml of 10% formalin. The pre-weighed

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[1] Although the taxonomic characterization of the fuel isolates was beyond the scope of this study, the more obvious physical and cultural characteristics noted during the tests are shown in Appendix A.

3

filter with the mycelium on it was transferred to an aluminum weighing dish and dried "in vacuo" over anhydrous $CaSO_4$ for 7 days (to constant weight), weighed, and the growth determined as mg of mycelium per flask.

## III. Results and Discussion.

The membrane filtration technique gave reasonably adequate precision as indicated by the data depicted in Table I; therefore, this technique was used to determine microbial concentration of fuel samples.

TABLE I. Precision of the Membrane Filtration Method

| Organism Type [1] | Sample Type | No. of Replicates | Sample Size, ml | Colony Count ±S.D. | Coefficient of Variation, % |
|---|---|---|---|---|---|
| B | Aqueous Suspension | 20 | 1.0 | 240±5 | 5 |
| B | " | 20 | 1.0 | 19±2 | 11 |
| B | " | 10 | 1.0 | 148±7 | 5 |
| F | " | 6 | 1.0 | 38±2 | 5 |
| F | " | 4 | 1.0 | 342±17 | 5 |
| B | Fuel Suspension | 20 | 500.0 | 103±9 | 9 |
| B | " | 10 | 100.0 | 17±2 | 12 |

[1] B = Bacteria, F = Fungi

The results shown in Table II illustrate that Triton X–100 either has no significant effect on microbial growth, or, if it does, a rinse with 100 ml of phosphate buffer is adequate for complete removal of any residual detergent; therefore, a 100 ml rinse was selected as standard procedure. Data in Table III illustrate that the settling rate of microorganisms in jet fuel is a very significant factor in determining a time for sampling fuel from the aircraft. Microbial suspension caused by refueling, flight, or other tank turbulence can seriously affect field tests for fuel contamination. Table III shows the number of bacteria present 4 inches below the surface of a laboratory tank up to 4 hours after agitation.

TABLE II. Effect of Detergent Rinse on Bacterial Growth Rate

| Volume Buffer Rinse (ml) | No. of Organisms per Cultured Plate[1] | |
|---|---|---|
| | E. coli | B. subtilis |
| 0 | 129 | 32 |
| 25 | 133 | 31 |
| 100 | 136 | 32 |
| 500 | 140 | 28 |

[1] Each figure represents the mean of three individual plate counts.

TABLE III. Concentration of Bacteria Four Inches Below Surface of Fuel at Various Time Intervals After Suspension

| Time After Shaking (min) | Bacteria/25 ml |
|---|---|
| 0 | 1,000 |
| 5 | 1,000 |
| 10 | 1,000 |
| 20 | 1,000 |
| 30 | 636 |
| 100 | 431 |
| 240 | 400 |

That microorganisms can distribute themselves throughout the fuel under static conditions is illustrated in Table IV. After 8 days' incubation, considerable numbers of bacteria, which were originally introduced into the water layer only, were noted up to 14 inches above the fuel-water interface; therefore, the organisms should have no particular difficulty in contaminating any new water pockets introduced by refueling or condensation.

TABLE IV. Vertical Distribution of Microorganisms in Fuel-Water Systems After Standing for Eight Days

| Tank No. | Distance Above Fuel-Water Interface (inches) | Bacteria/liter | Fungi/liter |
|---|---|---|---|
| 2 | 14 | 20 | 20 |
| | 7 | 320 | 0 |
| | 1 | 20 | 0 |
| 3 | 14 | 70 | --[b] |
| | 8 | 9270 | --[b] |
| | 2 | 3790 | --[b] |
| | −0.25[a] | $2.7 \times 10^{10}$ | --[b] |
| 4 | 14 | 120 | 0 |
| | 7 | 20 | 0 |
| | 1 | 1320 | 0 |

[a] Sampling of Water Layer below interface.
[b] Fungi were not counted separately in tank #3; counts shown represent the total number of colonies which grew on nutrient agar.

While DeGray and Killian[7] and others have established qualitatively the ability of microorganisms to remain viable in essentially "dry" fuel, we were interested in determining whether a population increase might occur under these conditions. The results of this experiment are shown in Table V. Neither bacteria nor fungi multiplied appreciably without the presence of a discrete water phase; however, considerable numbers of both remained viable for the period of the experiment.

4

TABLE V. Survival of Microorganisms in Fuel Phase

| Time (days) | Bacteria/liter | Fungi/liter |
|---|---|---|
| 0 | 18 | 1 |
| 9 | 20 | 1 |
| 17 | 10 | 2 |
| 24 | 7 | 1 |
| 63 | 8 | 2 |

Since absolutely anhydrous jet fuel is impractical to maintain in field refueling operations, growth or survival of microorganisms under these conditions was not studied.

In fuel-water systems, Biobor-JF appears to inhibit fungi very effectively. Table VI indicates that this biocidal action occurs within the first four days of exposure.

There was no appreciable bacterial inhibition in the water layer under the conditions of constant Biobor concentration and the fuel/water ratio existing in these laboratory experiments.

TABLE VI. Biobor-JF Inhibition In Fuel-Water Systems[1]

| Tank Number | Fuel Layer Bacteria/ liter | Fungi/ liter | Water Layer Bacteria/ ml | Fungi/ml |
|---|---|---|---|---|
| Zero Time[2] | | | | |
| 1 (control) | 10 | 30 | $4.0 \times 10^6$ | $1.2 \times 10^3$ |
| 2 (control) | 120 | 30 | $4.0 \times 10^6$ | $1.1 \times 10^3$ |
| 3 (Biobor) | 40 | 30 | $4.0 \times 10^6$ | $1.3 \times 10^3$ |
| 4 (Biobor) | 20 | 40 | $3.0 \times 10^6$ | $1.1 \times 10^3$ |
| 4 Days | | | | |
| 1 (control) | 0 | 9,800 | $3.23 \times 10^4$ | $2.0 \times 10^2$ |
| 2 (control) | 20 | 7,200 | $6.1 \times 10^9$ | $1.0 \times 10^2$ |
| 3 (Biobor) | 10 | 0 | $3.1 \times 10^9$ | 0 |
| 4 (Biobor) | 40 | 0 | $3.4 \times 10^9$ | 0 |
| 20 Days | | | | |
| 1 (control) | 2,200 | 2,700 | $4.8 \times 10^8$ | $7.4 \times 10^3$ |
| 2 (control) | 2,500 | 2,500 | $2.0 \times 10^8$ | $9.5 \times 10^3$ |
| 3 (Biobor) | 20 | 0 | $8.0 \times 10^8$ | 0 |
| 4 (Biobor) | 20 | 0 | $9.0 \times 10^8$ | 0 |
| 33 Days | | | | |
| 1 (control) | 1,510 | 2,600 | $3.28 \times 10^8$ | $2.0 \times 10^4$ |
| 2 (control) | 1,600 | 2,900 | $7.57 \times 10^8$ | $1.27 \times 10^4$ |
| 3 (Biobor) | 20 | 0 | $6.0 \times 10^8$ | 0 |
| 4 (Biobor) | 20 | 0 | $4.0 \times 10^8$ | 0 |

[1]Each tank contained 1950 ml of jet fuel and 50 ml of Bushnell-Haas salts solution. Tanks 3 and 4 contained Biobor-JF in the fuel fraction at a concentration of 270 ppm by weight; this concentration corresponds to 20 ppm of elemental boron.
[2]Zero time—samples were removed just prior to the addition of Biobor-JF to Tanks No. 3 and 4.

After the 33rd day, accurate sampling of the water phase became impossible due to the formation of fungal mats in the control tanks. Despite the lack of fungal counts in the treated tanks, two small fungal colonies (white) appeared in the water layer of tank #3 and grew slowly to approximately 1 cm in diameter.

The complete absence of viable fungi in the treated laboratory tanks on the 4th day is interesting. Both phases remained free of viable fungi for the remainder of the experimental period although the fuel-water ratio was only 39:1.

With this fuel-water ratio, the quantity of Biobor-JF present would give a concentration in the water layer of approximately 10,000 ppm at equilibrium. The greater fuel-water ratio existing in an aircraft fuel tank would permit a much higher Biobor-JF concentration to build up in the static water phase.

Inhibition by Biobor-JF was less marked when the organisms were incubated on a solid medium. Uniformly-inoculated petri dishes of nutrient agar showed no zone of inhibition around a Biobor-JF-saturated paper disc after 48-hours' growth with the organisms tested. Good growth of all 8 bacterial isolates tested was obtained on nutrient agar containing 400 ppm of Biobor-JF; 3 of 5 fungal isolates showed profuse growth on Sabouraud Dextrose Agar at 72 hours with the same Biobor-JF concentration.

Of the four bacterial isolates tested on solid medium incorporating 4,000 ppm Biobor-JF as the sole source of carbon, all exhibited growth after 3 days at 34°C. On the 10,000 ppm Biobor-JF medium, three of the four isolates showed a slow, steady and eventually profuse growth after one month at room temperature (26°C). Of these three isolates which grew on 10,000 ppm, however, two were also capable of growing on a solid medium containing only inorganic salts; therefore, they must be capable of using atmospheric $CO_2$ as a carbon source. The remaining isolate of the three capable of growth on 10,000 ppm could not grow on the salts medium; therefore, it must have been utilizing the Biobor-JF as a source of carbon. None of the fungal isolates was capable of growth in the presence of even the lowest concentration of Biobor-JF when no other carbon source was present.

Growth inhibition produced by various concentrations of Biobor-JF was more apparent in broth cultures. These results are shown in Table VII.

TABLE VII. Effect of Biobor-JF on Bacterial Growth in Liquid Culture

| Biobor-JF Concentration (ppm) | Optical Density at 600 mμ | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Known Species[3] | | | | Fuel Isolates | | | | | | |
| | E. coli | A. aerogenes | B. subtilis | S. marcescens | #10 | #11 | #13 | #14 | #16 | #17 | #19 |
| 0 | 0.64 | 0.57 | 0.48 | 0.86 | 0.42 | 0.08 | 0.21 | 0.40 | 0.45 | 0.44 | 0.66 |
| 200 | 0.68 | 0.62 | 0.39 | 0.77 | 0.38 | 0.06 | 0.17 | 0.40 | 0.43 | 0.41 | 0.72 |
| 500 | 0.66 | 0.57 | 0.39 | 0.77 | 0.17 | 0.05[1] | 0.15 | 0.37 | 0.40 | 0.37 | 0.65 |
| 1,000 | 0.63 | 0.53 | 0.10 | 0.71 | 0.05 | 0.03[1] | 0.13 | 0.12 | 0.34 | 0.28 | 0.62 |
| 5,000 | 0.10 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.01 | 0.00 |
| 10,000 | 0.03 | 0.00[2] | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |

[1]Fuel isolate #11 showed a slight granularity in the broth culture at the 500 and 1,000 ppm concentrations.
[2]A. aerogenes showed a slight growth on the second day at the 10,000 ppm concentration.
[3]Pure cultures of Escherichia coli, Aerobacter aerogenes, Bacillus subtilis, and Serratia marcescens were generously supplied by Dr. L. Vernon Scott and his staff, Department of Microbiology, University of Oklahoma Medical Center, Oklahoma City, Oklahoma

Bacteria exhibited considerable species variation in their ability to grow in the presence of higher concentrations of the additive in liquid media. Whereas most of the organisms tested were completely inhibited at concentrations between 1,000 and 5,000 ppm, E. coli and Fuel Isolate #13 showed significant growth at the highest concentration used, 10,000 ppm. A. aerogenes exhibited a similar resistance to inhibition by Biobor-JF.

Generally, the fungi all respond to the same concentration ranges of Biobor-JF; however, resistant cultures were noted. The results of this experiment are shown in Table VIII.

TABLE VIII. Effect of Biobor-JF on Fungal Growth in Liquid Culture[1]

| Biobor-JF Concentration (ppm) | Milligrams of Mycelium/75 ml Growth Medium Fuel Isolate No. | | | | |
| --- | --- | --- | --- | --- | --- |
| | 1 | 2 | 3 | 4 | 6 |
| 0 | 155 | 454 | 425 | 135 | 78 |
| 200 | 159 | 389 | 352 | 120 | 55 |
| 500 | 139 | 391 | 0 | 114 | 51 |
| 1,000 | 58 | 289 | 367 | 71 | 48 |
| 5,000 | 0 | 283 | 0 | 0 | 46 |
| 10 000 | 0 | 7 | 0 | 0 | 15 |

[1]All flasks were incubated for 5 days at 26°C with intermittent agitation

Fungal isolates #2 and #6 were the least inhibited by Biobor-JF, showing only 37.5% and 41% inhibition respectively at the 5,000 ppm concentration. Isolate #6 grew to 19% of the control weight at 10,000 ppm.

Field tests were conducted concurrently with the laboratory testing in cooperation with the Engineering Division, Aircraft Services Base (Engineering Report on Jet Fuel Additive Biobor-JF for Biocidal Effects, Report #66-25, prepared by AC-884). Samples were collected from symmetrically-located control and Biobor-JF-treated tanks on a Convair 880 aircraft during routine operation over a 3-month period. Representative data from the aircraft fuel samples are shown in Table IX. These data proved to be inconclusive, partially due to the cold-weather conditions which inhibited growth even in the control tanks. This lack of microbial growth in the untreated fuel prevented significant comparisons to be made with populations in the Biobor-JF-treated tanks.

TABLE IX. Microorganisms in Fuel from Convair 880 during Field Tests

| Sampling Date | Bacteria/Liter | | Fungi/Liter | |
| --- | --- | --- | --- | --- |
| | Control Tank | Biobor-Treated Tank | Control Tank | Biobor-Treated Tank |
| 1-27[1] | 6 | 4 | 10 | 4 |
| 2-3 | 8 | 20 | 114 | 10 |
| 2-12 | 486 | 6 | 954 | 2 |
| 2-17 | 58 | 38 | 70 | 14 |
| 3-11 | 10 | 6 | 10 | 10 |
| 3-19 | 10 | 22 | 11 | 0 |
| 3-24 | 32 | 41 | 84 | 0 |
| 4-27[2] | 2 | 22 | 15 | 0 |
| 5-6 | 26 | 3 | 12 | 0 |

[1]Samples on this date were collected immediately prior to addition of the Biobor-JF (270 ppm).
[2]On 4-5-65, the aircraft was refueled with military JP-4 fuel containing 0.10-0.15% of the Phillips PFA-55MB deicing additive which is also known to exhibit marked biocidal effects. Refueling with JP-4 was continued during routine operation for one week. On 4-13-65, all tanks were drained and refilled with Conoco Jet-50 fuel; Biobor-JF was added to the #3 tank to a concentration of 270 ppm. Tests were continued in the usual manner until termination of the field project.

A comparison between the counting method used in this study and the SOHIO test method, (STM R-54-65-T), which utilizes an extraction of the fuel sample with nutrient broth, indicated that the methods gave comparable results provided the fuel contained no minute water droplets clinging to the sides of the container. The presence of these water droplets, which was noted in some field samples, would produce an erroneously high count by the SOHIO method.

Preliminary studies established a Biobor-JF distribution coefficient of approximately 440 where:

K(distribution coefficient) = $\dfrac{\text{Concentration of additive in water}}{\text{Concentration of additive in fuel}}$

or : K = $\dfrac{\text{Weight of additive in water}}{\text{Weight of additive in fuel}}$

provided the fuel and water volumes are equal.

The equation for calculating the concentration of additive in the water layer which is achieved by addition of a known amount of additive to a defined system is as follows:

$$C_{(water)} = \frac{K \cdot W_t}{V_f + K \cdot V_w}$$

where  K = distribution coefficient
$W_t$ = total weight of Biobor-JF in system
$V_f$ = volume of fuel
$V_w$ = volume of water

Computations based on these formulae indicate that the Biobor-JF concentration in the water layer of an aircraft fuel tank, under field conditions, would be expected to exceed the biocidal level of 5,000–10,000 ppm when the fuel is repeatedly treated at the 270 ppm maximum level recommended by SOHIO.

In conclusion, Biobor-JF appears to be an effective fungal inhibitor for jet fuels when used at the recommended concentrations. Continued use could eliminate fungi from any new water pockets formed in the fuel tanks from condensation or from cooling of water-saturated fuel. Our experiments indicate that Biobor-JF is a less effective anti-bacterial agent in fuel-water systems. Whether or not resistant strains of bacteria or fungi would develop and prove to be a problem in practical applications cannot be predicted from existing data. Further field studies, under conditions more favorable to microbial growth, are indicated.

# APPENDIX A
## CHARACTERISTICS OF FUEL ISOLATES

| *Culture Number* | *Characteristics* |
|---|---|
| 1 | Fungus: light-brown growth on SDA[1], colonies round and convex, no diffiusable pigment produced. |
| 2 | Fungus: profuse white growth on SDA turning light-grey with age, brown pigment diffuses into medium. |
| 3 | Fungus: long, white filamentous growth on surface, light-brown pigment diffuses into medium in old cultures. |
| 4 | Fungus: rich brown coloration, profuse growth on SDA with yellowish-brown pigment diffusing into medium. |
| 6 | Fungus: sparse, yellowish growth on SDA becoming greenish-brown with age, yellow fruiting bodies appear in old cultures. |
| 7 | Fungus: profuse, white, silky growth on SDA, colonies exude an opaque pigment into medium with age and surface growth becomes a greyish-pink in color. |
| 9 | Fungus: profuse, light-brown growth on SDA, raised, slightly-convex colonies with white edges, no diffusable pigment. |
| 9–B | Fungus: raised, green colonies on SDA with white edges. |
| 10 | Bacterium: rods growing in singles, pairs and chains, round ends, length about 2–4X width, gram-positive, non-acid-fast, forms pellicle in NB[2], colonies circular, convex, entire, moist, cream-colored on NA.[3] |
| 11 | Bacterium: large rods with rounded ends, mostly single rods showing granular staining, gram-negative, forms pink pellicle in NB with some growth in depth; round, convex, moist, pink colonies on NA. |
| 12 | Bacterium: rods, mostly long chains with much branching, gram-positive, granular growth in NB, profuse white colonies with convoluted surfaces on NA. |
| 13 | Bacterium: small rods, short, almost coccoid in shape, gram-negative, motile, shows uniform, abundant growth throughout medium in NB; colonies on NA are circular, raised, entire, white, moist, and translucent. |
| 14 | Bacterium: rods, rounded ends, length approximately 6X width, singles, pairs, long chains, gram-positive, non-acid-fast, forms slight pellicle in NB with heavy flocculent growth in depth. Forms irregular, raised, undulate, opaque, white colonies with translucent centers on NA. |
| 16 | Bacterium: rods, singles, pairs and short chains, gram-positive, non-acid-fast, produces slightly granular, white pellicle in NB. Colonies on NA are circular, convex, entire, ivory-colored and opaque. |
| 17 | Bacterium: rods with rounded ends, singles, pairs and chains, gram-positive, non-acid-fast, forms white pellicle in NB, colony appearance same as culture #16. |
| 18 | Bacterium: rods forming long chains, gram-positive, does not stain easily with aqueous stains; colonies on NA are roughly circular, umbonate, with undulate edges and considerable yellowish growth below surface of medium; colony has hard, white surface becoming rough with age; small circular, umbonate, white colonies grow on surface of NB but do not grow in depths. |
| 19 | Bacterium: short rods, singles, pairs, short chains; have rounded ends, motile, gram-negative, forms slight white pellicle in NB with white clumps growing in depths; has white, convex, slightly rough colonies on NA. |

[1] SDA—Sabouraud's dextrose agar
[2] NB—Nutrient broth
[3] NA—Nutrient agar

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