



Homeland Defense & Security  
Information Analysis Center



# HDIAC TECHNICAL INQUIRY (TI) RESPONSE REPORT

## Bacteria and Fungi Contamination in Fuel and Hydraulic Oil

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## About

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### TI Research

A chief service of the U.S. Department of Defense's Information Analysis Centers is free technical inquiry (TI) research limited to four research hours per inquiry. This TI response report summarizes the research findings of one such inquiry. Given the limited duration of the research effort, this report is not intended to be a deep, comprehensive analysis but rather a curated compilation of relevant information to give the reader/inquirer a "head start" or direction for continued research.

## Abstract

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The Homeland Defense and Security Information Analysis Center (HDIAC) received a technical inquiry regarding microbial contaminants of the fuel and hydraulic liquid in aircraft. A particular focus was on all known microbes detected in the fuel and hydraulic liquid in aircrafts or any other similar systems, the enzymes these microbes use for their nutrition, morphological and physiological peculiarities of such biofilms, and any other related and relevant information. HDIAC subject matter experts were tasked to conduct a literature search and present findings from some of the most recent articles on this subject.

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## 1.0 TI Request

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### 1.1 Inquiry

What fungi and bacteria have been identified contaminating fuel and hydraulic fluid?

### 1.2 Description

The inquirer's experience with three separate cases working with microbes/contaminants of fuel and hydraulic liquid in aircrafts shows that, in each case, various microbial communities are involved in the contamination and there is no common pattern in their species composition. A common morphological feature in all three cases is building up the microbial biofilm consisting of two or more fungal species, with or without bacteria. Another common location feature is that those biofilms are located on the bottom of the fuel/liquid's tank, growing in the thin water layer between the tank bottom and its contents. An additional common feature is that the biofilms damage the contents of the tank, as well as cause the corrosion of its surface. The inquirer is very interested in all known microbes detected in the fuel and hydraulic liquid in aircrafts or any other similar systems, the enzymes these microbes use for their nutrition, morphological and physiological peculiarities of such biofilms, and any other related and relevant information.

## 2.0 TI Response

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Subject matter experts at Texas Research Institute Austin conducted a literature search on occurrences of bacterial/fungal infestation of fuel/hydraulic systems.

To thrive, bacteria/fungi need:

- Water: emulsified in the petrochemical product, 500 ppm (0.5 ml per liter of oil) are sufficient
- Food: carbon, nitrogen, and phosphorous, readily supplied by the petrochemical product
- Oxygen: air is typically present in fuel/oil on the order of 7% to 10% by volume
- Optimum temperatures: 24 °C to 49 °C (75 °F to 120 °F)
- Dark, Stagnant, or Low-Flow Areas: the reservoir in hydraulic systems or unstirred, stored fuel
- Suspended Particles: assist with initial transportation and colonization

While all of these ingredients are required to sustain bacterial growth, water is the key.

## 2.1 Fuel Contamination

Fuel contamination identification depends on the type of bacteria and the conditions available for access to water and food within the fuel.

### 2.1.1 Background

Microbial growth (MBG) may occur wherever any water accumulates in aviation fuel tanks and systems. Housekeeping measures for aviation fuel focus on the regular removal of water, such as daily draining of airport storage tanks and filter vessels. The presence of water allows heavy MBG to occur, and fuel quality can be affected due to particulate contamination of fuel with microbial biomass and contamination with by-products of MBG such as biosurfactants and sulfide. Although fuel specifications, such as American Society for Testing and Materials (ASTM) D1655 [1] and Defense Standard (DEF STAN) 91-091 [2], generally do not include specific limits for microbial contamination, in severe cases, MBG can cause fuel to fail specification and there may be serious disruption of supply. If microbiologically contaminated fuel is uplifted onto aircraft, there is a possibility for serious operational problems.

Consequently, industry guidance from the International Air Transport Association (IATA), Joint Inspection Group, ASTM, and Airlines for America places a strong emphasis on preventing MBG in the fuel supply chain and in aircraft fuel tanks before it can cause operational problems. An important part of this is to regularly monitor for the presence of microbial contamination.

Because biocides are usually not permitted to be added to fuel in the aviation fuel supply chain, where contamination can get out of control, remediation can require extensive system downtime for physical cleaning and/or downgrading of product. Selected biocides can be used to treat aircraft tanks under carefully controlled conditions (after checking the aircraft maintenance manual), but this can also lead to extended aircraft downtime and potential disruptions to flight operations. Other common problems include disarming of filter water separators, fuel tank corrosion, clogging of engine fuel filters, and malfunction of fuel quantity indicator systems.

MBG consists of living micro-organisms (also called microbes) growing at a fuel and water interface. They may be yeast, fungi, or bacteria. Fungi is a major source of problems in fuel. This type of contaminant appears as a stringy, web-like substance, usually grey or brown in color. MBG testing in fuel focuses on bacteria, yeast (a subgroup of fungi), and fungi (mainly filamentous fungi *Hormoconis resinae* [H.res]). Fungi are simple aerobic organisms and may grow to form fungal mats. Fungi produce spores that are like seeds that germinate in the

presence of water. Once a spore germinates in water, a fungus grows by using fuel for food, along with trace materials in the water and dissolved oxygen.

A wide variety of microorganisms may enter fuel systems via air, sea, fresh water, soil, or other means. The fuel/water interface is an ideal place for fungi and bacteria to grow. The exact mechanisms of sludge and slime formation and corrosion from bacterial and fungal growth are complex. In the right environment, bacteria and fungi may grow and result in ground fuel system problems such as but not necessarily limited to:

- Coalescer Element Spotting
- Clogging and/or Disabling
- Dark-Colored Water Bottoms or Smelly “Black Water”
- Fuel Tank Corrosion (in extreme cases)

In aircraft, microbial contamination of fuel may lead to:

- Fuel-Level Gauge Malfunctions
- Fuel System Clogging
- Fuel Tank Corrosion

H.res is damaging in fuel because of several reasons:

- **Size and Bulk:** When compared to yeasts and bacteria, H.res produces far more biomass and is thus more likely to cause blockage problems.
- **Corrosive Effects:** The by-products of microbial metabolism from H.res, along with other microorganisms, may be acidic and can induce harmful corrosion in aircraft fuel tanks.
- **Staying Power:** Because of the way H.res grows between fuel and water, it usually starts on small water droplets and then covers the droplet, holding it in place. It then continues to grow, generating more water due to its metabolism. As part of the process, it firmly attaches itself to the tank, staying even after the water is drained.

Microbially influenced corrosion (MIC) is also a realistic threat (the time frame can be weeks in some cases) from microbial infections where metal structures are used to store fuel. The by-product is a sludge-like substance that, in sufficient quantity, can cause corrosion on steel and aluminum surfaces and attack rubber fuel system components. It can also foul filters and system instrumentation. An operator in Asia was forced to write off two narrow-body jets after the effects of MIC took hold and costs of replacing an entire wing structure were prohibitive.



## 2.1.2 Identification

Microbial contamination in jet fuels is typically detected through colony-forming unit (CFU) tests, adenosine triphosphate (ATP) tests, or immunoassay-based antibody tests.

Testing for the presence of MBG material in fuel systems can be performed using the various commercial test kits (see IATA “Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks” [3]). Testing is done on both the water phase, if any, and on the fuel phase when microbial contamination is suspected. There are different technologies applied to testing such as traditional methods called CFU tests, methods using ATP technology, and the most modern antibody test technology. Still valid, these tests are often done off site, so the samples must be transported and tested within 24 hours. CFU tests must be incubated, which is an additional cost, and results are typically available within 4–8 days. ATP is a more modern but highly scientific test that gives more rapid results but is generally performed in laboratories and requires expensive hardware (called a luminometer) to furnish results. Both CFU and ATP tests detect general microbial presence either directly or indirectly, at least as much as can be grown or detected from the sample, and can be cross-contaminated by microbes present all around as well. Results can be higher than the actual conditions in the tank at sampling due to these factors, with risk of unnecessary maintenance, downtime, and costs being higher than required.

Immunoassay antibody tests are done on site and on the spot and require little training and no special equipment. Results can occur within 10–15 minutes in an easy-to-interpret format.

## 2.1.3 Research Work

Research into bacterial fuel contamination has been performed for many years and continues to be performed [4–6]. Recently, researchers have begun doing genomic investigations into the kinds of bacteria within fuel tanks [7, 8].

With the recognition of the dangers of microbial contamination, several studies have been undertaken regarding microbial contamination in aviation fuel from storage tanks [9–11] but few studies have examined fuel from aircraft fuel tanks [9, 10].

However, high microbial biomass has been detected in aircraft fuel tanks [10]. In addition, a B-52 crash was directly attributed to the plugging of the filtration system by microbial contamination in 1956 [12]. Also, a microbial attack caused aircraft fuel tank corrosion in an aircraft in Australasia in 1961 [13]. These examples show that microbial contamination in aircraft fuel tanks cannot be ignored.

## 2.2 Hydraulic Fluid Contamination

Hydraulic fluid contamination occurs similarly to fuel contamination and again depends on the bacteria strain, the availability of water, and the type and availability of food.

### 2.2.1 Background

Contamination of a hydraulic system by bacteria and fungi is rarer than in fuel systems. More often, hydraulic fluid is contaminated with metallic particles (a result of metal-on-metal contact), air (causing the fluid to look foamy), water, other chemicals (this often occurs as a result of the natural degradation of hydraulic fluid), and ingressed particulates (the result of particles that make their way into the fluid system from the outside, such as dust, mud, dirt, and sand).

*Pseudomonas oleovorans* prefer oil as a food source and therefore tend to grow rapidly in machines that leak substantial amounts of lubricating and hydraulic oil. Consequently, everything should be done to reduce such oil leakage but, if it cannot be prevented, such oils should be skimmed off the surface or centrifuged from the fluid. *Pseudomonas aeruginosa* can live on practically anything: minerals in the water, coolant concentrate, discarded food, or oils. Note that *pseudomonas oleovorans* and *pseudomonas aeruginosa* are both aerobic and facultative and are the two species present in all water miscible fluids. Of all known bacteria, they are also two of the most difficult to kill.

### 2.2.2 Research and Work

Instances of hydraulic fluid contamination are difficult to track. Anecdotal cases have been discussed for marine fuel oil systems (which are more susceptible to water contamination). One possible reason for the lack of information on hydraulic oil contamination may be the operating temperatures for hydraulic fluids. Fuel is stored (usually not heated) and used (combusted). Hydraulic oil is constantly reused and heated in hydraulic oil systems, meaning that this fluid is exposed to temperatures in the range of 50–65 °C for long periods of time, which can dampen or prevent large quantities of bacterial growth.

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## Biography

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