# Antimicrobials for Water Systems in Manned Spaceflight – Past, Present, and Future Applications and Challenges

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**The use of antimicrobials to control microbiological growth in manned spaceflight water-based systems has and will continue to have a unique set of challenges and needs. The challenges are varied, and include antimicrobial effectiveness, crew health and safety, materials compatibility, optimal system functionality, antimicrobial shelf life, means to monitor antimicrobial concentration, and means to re-introduce biocides periodically in the case of depletion. Needs vary from application to application, and include control of pathogens for crew health, control of biofilm formation for optimal system functionality, inhibition and prevention of microbiologically influenced corrosion, optimization of wetted metallic material life, and general living quarter and consumable aesthetics with respect to odor and taste. This paper outlines and discusses the various antimicrobials used in prior and current manned spaceflight water-based applications with focus on pros, cons and lessons learned. Design factors such as minimum inhibitory concentration, minimum lethal concentration, required circulated concentrations, materials selection, means to introduce, means to monitor real-time, and concentration maintenance are discussed. The challenges associated with longer term missions, as well as long-term system dormancy as envisioned for exploration missions, lunar habitats, and a manned Mars mission are outlined with respect to anticipated needs and potential design solutions.**

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## Nomenclature

*ACTEX* = Activated Carbon / Ion Exchange
*ALCLR* = Airlock Cooling Loop Recovery
*BFA* = Biocide Filter Assembly
*BMP* = Russian Micropurification Unit
*CCAA* = Common Cabin Air Assembly
*CFU* = Colony Forming Units
*CHX* = Condensing Heat Exchanger
*CM* = Command Module
*CWC-I* = Compatible Water Container - Iodine
*EATCS* = External Active Thermal Control System
*ECLSS* = Environmental Control and Life Support System
*EFSF* = EMU Feed-water Supply Filter
*EMU* = Extravehicular Mobility Unit
*EVA* = extravehicular activity
*2-FBA* = 2-formylbenzoic acid
*IATCS* = Internal Active Thermal Control System
*ISS* = International Space Station
*L* = liter
*LCG* = Liquid Cooling Garment
*LM* = Lunar Module
*LWC* = Liquid Cooling Garment Cooling System
*MCV* = Microbial Check Valve
*mg* = milligram
*MLS* = Mostly Liquid Separator
*NASA* = National Aeronautics and Space Administration
*OGA* = Oxygen Generator Assembly
*OPA* = ortho-phthalaldehyde
*ppm* = parts per million
*PWR* = Potable Water Reservoir
*PWS* = Potable Water System
*SDC* = silver dihydrogen citrate
*SKV* = air conditioner
*SRV-K* = system for water recovery from humidity condensate
*SSP* = Space Station Program
*TOC* = total organic carbon
*µS* = microsiemens
*WPA* = Water Processor Assembly

## I. Introduction

THE need for microbial control in water systems in everyday life is critical to the health of the users and to the proper functionality of the systems. Entire industries have been built to provide the chemicals and equipment to meet this need. Microbial control is taken for granted in most industrialized countries and is considered routine. Providing the same for water systems in manned spaceflight has a unique set of challenges not apparent to many.

The selection and use of antimicrobial agents in manned space-flight applications has a long and varied history. The early, pre-Space Shuttle missions, with the exception of Skylab, were relatively short-term with heavy reliance on launched expendables such as water, food and oxygen. Water-based, closed-loop hardware such as that found in internal thermal control systems were active for relatively short periods of time. Efforts to recycle or regenerate expendables on-orbit were minimal and the integration of Environmental Control and Life Support Systems (ECLSS) to "close the life support loop" was limited. The vehicles and associated hardware were returned to the ground and were either reworked for future use or retired from use. Antimicrobial selection criteria was appropriately geared towards key factors for short-term use such as crew health, toxicity, off-gas characteristics, flammability, stability, effectiveness for the application, maturity of use, program/user acceptability, and short-term materials compatibility.

During the later stages of the Space Shuttle Program, and into the Space Station and Orion eras, much more demand was placed on hardware needs, particularly in the area of use duration. Requirements for hardware functional life increased from days and weeks, to months and years. Systems and components were developed for new applications such as urine processing, humidity collection, water recycling, oxygen generation, and carbon dioxide / hydrogen reactivity for water generation. ECLSS systems were integrated in an effort to “close the life support loop” so the proper operation of one system would rely on the proper operation of interfacing systems. Additional factors for antimicrobial selection, above-and-beyond those already mentioned, came into play such as the long-term cumulative effect on the human user, long-term materials compatibility related to microbiological influenced corrosion, biofilm formation and fouling, ability to monitor the biocide, methods to add additional antimicrobial if it degraded and/or reacted, microbial resistance over time, and microbial mutation over time. As the duration of missions continues to increase, and as factors such as long-term system dormancy come into play, the previously cited antimicrobial selection factors are expected to become more important, and additional factors may come into play.

The purpose of this paper is to review the various antimicrobials used in prior and current manned spaceflight water-based applications with focus on pros, cons, and lessons learned. It is intended that this review of antimicrobials use in manned spaceflight water-based applications will provide the reader a foundation to build on for future, more demanding applications.

## II. Pre-Space Shuttle Era Antimicrobials

Prior to the Gemini missions, potable water was supplied to the crew using a flexible water pouch from which the astronaut would consume directly.[1] The water was supplied from the Cocoa Beach potable water system with no additional disinfectants added.[2] However, as inflight water systems became more complicated, the need for additional disinfectants became necessary. During the Gemini Program, the overall water system design required an interconnection between the potable water and the humidity condensate systems during contingency operations.[1] The risk of cross-contamination was further exacerbated by an existing interconnection between the humidity condensate and urine management system.[1] Taken together, the decision was made to add chlorine to the water loaded into Gemini prior to launch.

As with the Gemini Program, the use of potable water disinfectants continued with the Apollo Program. The Apollo Command Module (CM) water system interconnected the potable water supply with the humidity condensate creating a need for a chlorine disinfectant.[1] One key difference between the Apollo Command Module and Gemini water systems was that the CM relied on water produced by the vehicle fuel cells.[2] This input of untreated water reinforced the need for active addition of the disinfectant by the crew to maintain a residual level of at least 0.5 mg/L.[1] This concentration was maintained during Apollo 7 through 13 by adding 22 mL of a sodium hypochlorite stock solution (5000 mg/L available chlorine) every 24 hours. For Apollo 14 and thereafter, the disinfectant regimen was changed to every 24 hours adding both 22mL sodium hypochlorite stock solution (1860 mg/L available chlorine) and 22 mL of a mixture of 0.297 mol/L sodium dihydrogen phosphate and 0.217 mol/L sodium nitrate to extend the life of the chlorine in the system.[1]

The Apollo Lunar Module (LM) had an independent water system with tanks loaded with water and disinfectant prior to launch. Molecular iodine ($I_2$) was selected to replace chlorine as the disinfectant due to concerns about corrosion of the sintered nickel sublimator plates.[2] Iodine addition to the prelaunch water was targeted to maintain a minimum residual level of 0.5 mg/L; however, the effective concentration of iodine was expected to degrade over the course of the mission.[1] To mitigate this loss, depletion rates were estimated based on preflight measurements and a bacterial filter was installed when the data indicated that the iodine concentration had fallen below 0.5 mg/L.

The Skylab water system was a distinct departure from those used on relatively short term spaceflight missions. As a component of a long-term habitat, the water system had to provide water over the course of 3 separate missions within an 11 month period. Considering the long duration of the mission, a method of measuring the biocide levels over time was required. Iodine was selected as the disinfectant, as it was considered less reactive than chlorine, thus decreasing concerns about potential system corrosion or degradation of the biocide. Iodine was also a favored choice since simple starch-based tests were available to monitor iodine levels in the water. To maintain iodine at a biocidal concentration (5 to 6 mg/L), periodic injections of 75 mL of a 30 g/L iodine solution were added to the system. Since iodine has poor solubility in water, the solution was composed of 2 mol/L potassium iodide to every 1 mol/L iodine. While iodine disinfection worked exceptionally well during the 3 Skylab missions, maintaining iodine levels at biocidal concentrations in the water distribution system between missions was not possible, creating a need to drain the water from the distribution system between missions.[3]

## III. Space Shuttle Era Antimicrobials

### A. Potable Water and Extravehicular Mobility Unit (EMU)

Iodine was the primary antimicrobial used on the Space Shuttle Program with applications ranging from crew potable water to EMU cooling and feed-water loops. A device known as a Microbial Check Valve (MCV®), which consists of a canister containing polyiodide anions bound to quaternary amine fixed charges of a polystyrene-divinylbenzene copolymer anion exchange resin, was used for the addition of the iodine antimicrobial to the potable water. The bound polyiodide anions release biocidal iodine ($I_2$) in a controlled fashion to water that flows through the MCV.[4]

On-board the Space Shuttle, high purity water, produced as a byproduct of the fuel cells, would flow through an MCV canister which provided both a contact microbial kill and an elemental iodine residual of 0.5 – 4.0 mg/L (based on the life of the MCV resin and water flow-rate). MCVs were installed in the Shuttle Galley Auxiliary Port and the EMU Service and Cooling Umbilical for microbial control (Figure 1).[4]

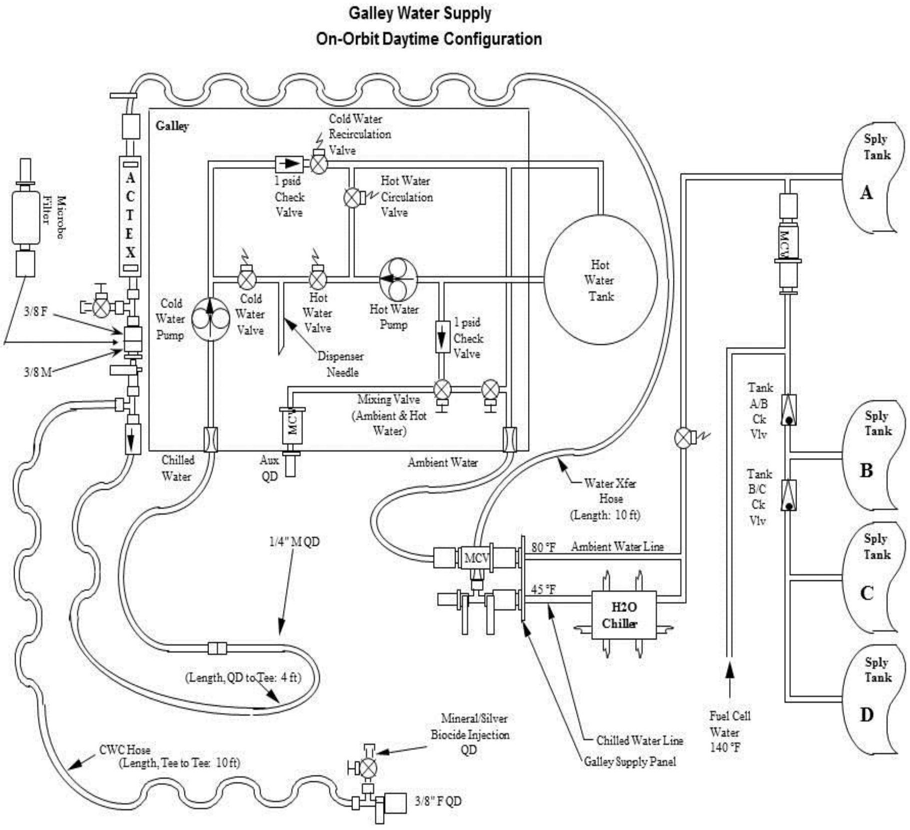


**Figure 1. MCV Location on the Space Shuttle Galley Water Supply System**

Once $I_2$ is added to water, it hydrolyses in a pH-dependent manner to form hypoiodous acid (HIO) and iodide ($I^-$). The overall stoichiometry of iodine hydrolysis in the pH 2 – 7 range is as follows:

$$I_2 + H_2O \leftrightarrow HIO + I^- + H^+$$

Like hypochlorous acid, HIO can deprotonate to form hypoiodite ($OI^-$) according to the following reaction:

$$HIO \leftrightarrow H^+ + OI^-$$

The various chemical species of iodine vary in antimicrobial power. The active antimicrobials are elemental $I_2$ and HIO. Other species that are either weaker antimicrobials or have no known antimicrobial properties include $I^-$, $IO_3^-$, and $OI^-$. Comparing the two antimicrobial-active iodine species, the oxidizing power of HIO is nearly twice that of $I_2$. The disinfection efficacy of the different iodine species depends not only on oxidizing potential, but also on penetration power. $I_2$ has higher penetration power than HIO.[5]

Previous studies have shown that iodine concentrations in water of lesser purity than that for the Space Shuttle, in the range of 5 – 10 mg/L, were very effective for many different types of microorganisms within 10 minutes at room temperature. Organisms that have been evaluated for iodine antimicrobial activity include enteric bacteria, amoebic cysts, and viruses. Overall, different microorganisms have different susceptibilities to iodine. Vegetative bacteria tend to be the most sensitive, whereas viruses have an intermediate sensitivity, and protozoa tend to be more resistant. Additionally, to different extents $I_2$ and HIO contribute to the disinfection effectiveness against different microbial types. Chemical speciation of the iodine compounds in water is highly pH dependent.[5]

Long-term testing with human subjects in the 1990s raised concerns about excessive iodine consumption and the potential for thyroid function changes over extended use. A NASA-convened panel of independent experts determined that the maximum safe iodine consumption for astronauts is on the order of 1mg total iodine per day, with no more than 0.5-mg/day from either food or water. As a result of this information, steps were taken to limit uptake of iodine starting with Shuttle flight STS-87 in November of 1997.[6]

Existing hardware, designed to remove iodine from Shuttle water to the MIR Space Station, was utilized for the iodine removal from the Shuttle Galley potable water. The driver for the Shuttle / MIR iodine removal effort was to ensure that iodine from the Shuttle potable water would not mix with silver in the MIR potable water and form a precipitate. The hardware used for this task utilized a packed cartridge of mixed anion/cation removal ion exchange resin and activated carbon.

The iodine removal cartridge (designated as the Activated Carbon / Ion Exchange (ACTEX) cartridge) was utilized to completely remove all forms of iodine from the Shuttle Galley potable water. A 0.2 µm bacterial filter was then added to the Shuttle Galley chilled water loop due to the elimination of residual iodine in the effluent water. Iodine, however, still remained in the hot water, which resulted in limiting the crew to only 12-ounces of hot water per day, or the equivalent of rehydrating two food items. Any other dehydrated food would then need to be rehydrated with deiodinated cold water, then heated as needed in the Shuttle Galley oven.[7]

The use of iodine in the previously described Space Shuttle applications has not resulted in materials compatibility issues of significance. Iodine continues to be used in several International Space Station (ISS) applications, with the heritage lack of material compatibility issues and long-term efficacy being strong drivers for continued use.

A short-fall of the use of iodine as an antimicrobial in both EMU water loops is the fact that the active forms of iodine are reduced over time due to absorption into non-metallic materials, to a lesser extent adsorption onto metallic surfaces and reduction to non-active iodine species over time. The available data suggests that biocidal levels of iodine in both loops last a matter of weeks depending on the operation of the loop. The iodine disinfection of both EMU water loops can best be described as a periodic disinfection that keeps microbial activity to a level that can be well tolerated in each of the two water loops.

## IV. ISS Era Antimicrobials

### A. Water Processor Assembly

The ISS Water Processor Assembly (WPA) produces potable water from humidity condensate and urine distillate.[10] Waste water is pushed from the waste water tank into the Mostly Liquid Separator (MLS) where gas is removed and passed through the Separator Filter to remove odor-causing contaminants. The water is pumped through the Particulate Filter followed by two Multifiltration Beds where non-volatile organic and inorganic contaminants are removed. Next, the process water enters the Catalytic Reactor where low molecular weight organics, not removed by the adsorption process, are oxidized in the presence of oxygen, elevated temperature, and a catalyst. A regenerative heat exchanger is used to recover heat from the effluent of the Catalytic Reactor to increase efficiency. The Reactor Health Sensor monitors the conductivity of the reactor effluent. The Ion Exchange Bed removes dissolved products of oxidation and adds iodine for microbial control using polyiodide anions bound to quaternary amine fixed charges of a polystyrene-divinylbenzene copolymer anion exchange resin.[10] The water is stored in the Water Storage Tank prior to delivery to the Potable Water Bus for crew consumption and payloads. [10] Iodine and iodide are removed from the Potable Water Bus to prevent potential impacts to the crew by an ACTEX filter containing activated carbon and ion exchange resin.[11] Figure 2 is a simplified schematic of the WPA.[10]

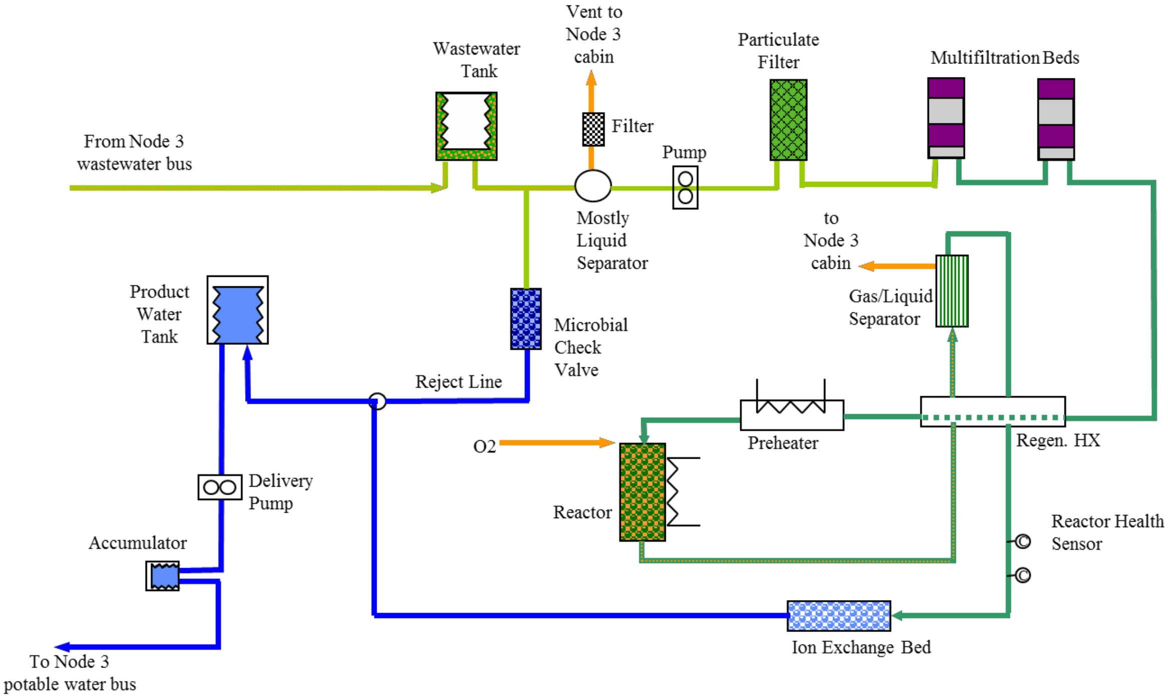


**Figure 2. WPA Simplified Schematic[10]**

The water processor assembly uses a combination of filtration, heat, and iodine to provide disinfection of microorganisms in the WPA. The Particulate Filter is a depth filter with a nominal rating of 0.5 µ and retains a large percentage of contaminates, smaller than their normal size rating, because of adsorption. In ground testing to document performance of the WPA, the particulate filter resulted in a 4 log reduction (10,000) between the Waste Tank and the Catalytic Reactor inlet.[12] The disadvantage is that microorganisms may reproduce within the filter matrix, penetrate deeper, and emerge on the downstream side in a phenomenon known as *grow-through*.[13] Any microorganisms passing through the Particulate Filter are exposed to elevated temperatures up to 267 ± 3°F for 10 minutes as the water passes through the Catalytic Reactor. In ground testing, the bacterial and fungal concentration was consistently less than 1 colony forming units (CFU)/100 mL.[12] Product water also contained less than 0.01 endotoxin units/mL and contained no bacterial DNA.[12] The Deionization Bed provides 1 – 4 ppm $I_2$ to ensure microbial control in the Water Storage Tank.

## B. Water Supply System in the Russian Modules of ISS

Humidity condensate in the Russian modules of ISS is collected by the system for water recovery from humidity condensate (SRV-K).[14] The condensate passes through a multifiltration bed containing activated charcoal, ion exchange resins, and a proprietary room-temperature catalyst to remove inorganic and organic contaminants by cationic exchange and oxidation. The catalyst provides removal of low molecular weight alcohols including ethanol and methanol. [14] An in-line conductivity sensor located downstream is used to determine if the water is of acceptable quality (less than150 µS/cm). Water that is acceptable flows through a conditioning bed, which adds magnesium, calcium, and other minerals, to enhance palatability. A silver ionizer is used to add silver (0.05 - 0.20 mg/L) for microbial control.[14] Factors such as pH and the presence of ions including chloride, nitrate, and sulfate can reduce the concentration of silver ions ($Ag^{+)}$ and lower the antimicrobial effectiveness.[13] $Ag^{+}$ also tend to plate out of solution onto system materials. Resistance of microorganisms to silver can occur by enzymatic conversion into less toxic forms, sequestration and binding in the cell wall, alteration of uptake pathways, and efflux systems to reduce intracellular concentrations.[13] Therefore, prior to dispensing the conditioned water for drinking, product water is pasteurized at 85°C to prevent growth of viable microorganisms by a regenerative heat exchanger and storage in a heated accumulator.  Hot water is available to the crew directly from the accumulator and cold water is provided by re-routing the hot water through the regenerative heat exchanger.

### C. Internal Active Thermal Control System (IATCS)

The main purpose of the IATCS aboard the ISS is removal of heat loads from payload and system racks. The IATCS is a water-based system which works in conjunction with the External Active Thermal Control System (EATCS), an ammonia based system, which is interfaced through a heat exchanger to facilitate heat transfer.[15]

The original antimicrobial selected was silver sulfate at a concentration of 0.1 – 3 parts per million (ppm).[15] Silver ion concentration rapidly decreased below detection limits within a few hours after circulation through system components. Silver ions in the coolant rapidly underwent an oxidation-reduction reaction with nickel at pH 9 to 10. Nickel acted as a reducing agent and contributed electrons to the silver ions to form silver metal. The reaction also increased the aqueous concentration of nickel ions. Repeated additions of the silver salt created short duration increases in corrosion rates of the nickel braze alloys in the cold plates and heat exchangers during the silver deposition process.[18] The reduction of silver was also accompanied by a 6 log increase in heterotrophic bacteria that potentially increased risk to crew health and safety and system performance.

Studies were conducted to select an antimicrobial to control microbial growth in the system based on requirements for disinfection at low chemical concentration (effectiveness), stability, material compatibility, low toxicity to humans, compatibility with vehicle environmental control and life support systems (ECLSS), ease of application, rapid on-orbit measurement, and removal capability.[15]  An aromatic dialdehyde compound, ortho-phthalaldehyde (OPA), was initially implemented at a concentration of 100 ppm OPA in the U.S. Lab on November 3, 2007.

#### 1. *Effectiveness*

Effectiveness of OPA was determined by a series of tests including minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) for planktonic microorganisms isolated from the IATCS at pH 9.0 and pH 9.5.  OPA at pH 9.0 had a MIC of 10 ppm and an MLC of 15 ppm.[15] A 6 log reduction of biofilms was achieved in 24 hours. The OPA MIC at pH 9.5 was 5 – 10 ppm but the MLC increased to 30 ppm for all microorganisms tested except *Methylobacterium extorquens* which was greater than 150 ppm.[15]

#### 2. *Stability*

Ground-based testing identified 3 modes of OPA degradation including oxidation reactions from base/metal catalysis, base-catalyzed reactions, and reaction with ammonia.[15] The major degradation product formed was 2-formylbenzoic acid (2-FBA), likely due to catalytic oxidation with Nickel 201 and Nickel braze alloys in the IATCS. Compound 2 in Figure 3 is 2-formylbenzoic acid.[15] Ammonia readily reacts with OPA to produce compounds 4 and 4’ in Figure 3. Compound 5 has also been detected in used IATCS fluids and can undergo a base catalyzed hydrolysis to Compound 6. Compounds 7 and 8 were detected at low levels, but their origin was not clear.

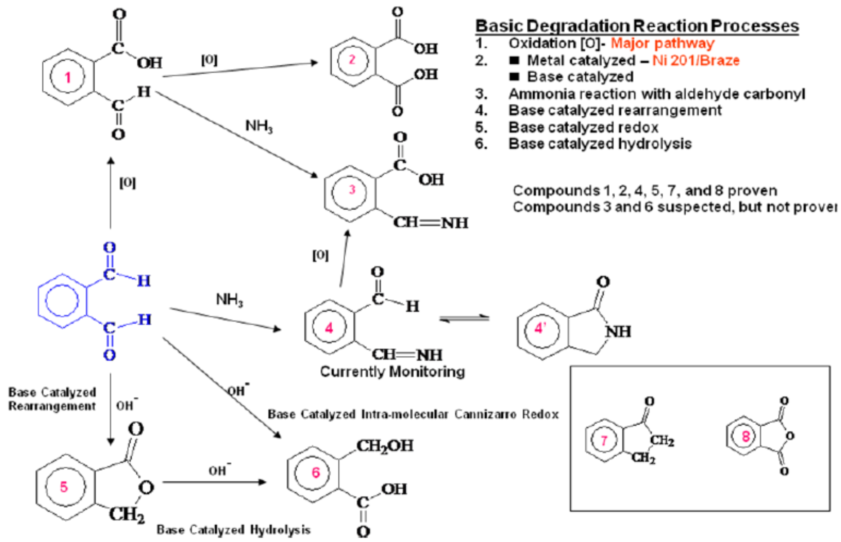


**Figure 3. Structures and Origin of OPA Reaction Products[15]**

#### 3. *Material Compatibility*

OPA, at up to 600 ppm, was compatible with non-metallic materials of construction including Nylon 11, Nylon 66, polypropylene, Valox® (polybutylene terephthalate), ethylene propylene rubber (EPR), epoxy highly filled casting material, and unfilled epoxy resin.[15] Nylons exhibited degradation in tensile strength, weight gain, and volume swell attributable solely to water absorption. OPA at up to 600 ppm was also compatible with metallic materials of construction including CRES 15-5 PH and 17-7 PH, Titanium 6-4, CRES 302, Hastelloy W weld material deposited on CRES 347, BNi-2 braze material deposited on CRES 347 and Ni-201 to simulate a parting sheet – fin heat exchanger configuration, BNi-3 braze material deposited on CRES 347 and Ni-201 to simulate a parting sheet – fin heat exchanger and cold plate configuration, and BNi-3 braze material deposited on CRES 347 with a Nioro (AMS 4787, BAu-4) repair to simulate a cold plate repair process.[15] BNi-3 braze and Nioro braze repaired BNi-3 were the most sensitive materials to OPA with an average corrosion rate of 0.12 mpy which doesn't impact hardware function and life.[15]

### 4. *Toxicity*

The toxicity of OPA was determined by NASA Johnson Space Center Toxicology Group. Circulated OPA up to 500 ppm was determined to be Toxicity Hazard Level (THL) 0 (Nonhazard) defined as slight irritation that lasts less than 30 minutes and doesn't require therapy for all toxicology parameters.[15] Initial effluent concentrations of OPA, greater than 1000 ppm, introduced into a payload by-pass stream from the antimicrobial applicator were determined to be a Toxicity Hazard Level 1 (Critical), defined as slight to moderate irritation that lasts more than 30 minutes and requires therapy due to irritation of soft tissues such as the eye. OPA loaded resin beads containing 0.25 g/cm$^2$ OPA in the antimicrobial applicator were assessed as a THL 0.[15] Evaluation of leaked coolant containing OPA, that dried as a film on surfaces, was determined to be a THL 0 for eye contact.[15]

### 5. *ECLSS Compatibility*

The impacts of OPA to ECLSS were based on the leakage specifications of the modules in the United States On-orbit Segment (USOS), Columbus Module, Japanese Experiment Module and the scrubbing capability provided by the U. S. Trace Contaminant Control System (TCCS), the Russian micropurification unit (BMP), and by humidity condensate absorption for a crew of three from the Common Cabin Air Assembly (CCAA) and Russian air conditioner (SKV). This conservative assessment limited circulating OPA to 75 – 105 ppm due to potential MF Bed breakthrough and impacts on the high temperature Catalytic Reactor in the WPA.[15] The actual leakage rate for all modules was only 7.08 cm$^3$/hr which was less than half of the specified leakage rate. The as circulated concentration of OPA was increased to 50 – 500 ppm through the management of risk by rapid leak detection, isolation, and OPA removal and neutralization.[15]

### 6. *Application*

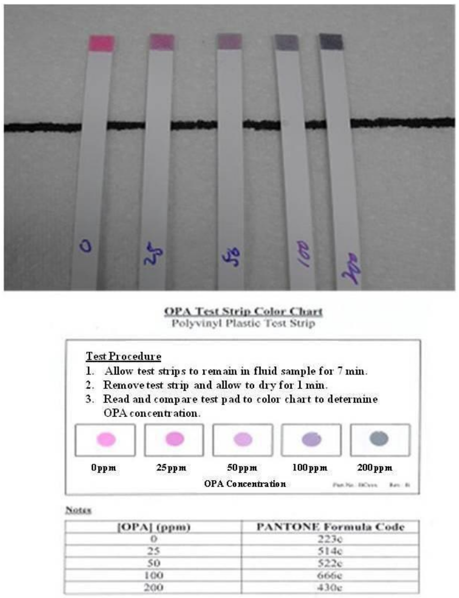
The method selected to safely deliver OPA to the IATCS coolant was immobilization of the active antimicrobial to a solid substrate. The immobilization process involved a solvent evaporation technique that allowed the OPA to be physically constrained in a porous resin material.[15] The OPA elution from the resin material into the coolant has been an accurate, reproducible, and safe method to limit crew exposure to concentrated levels of OPA.

### 7. *Measurement*

A rapid colorimetric test for OPA was developed using test strips containing a proprietary para-rosaniline indicator that changed color from pink to dark gray with increasing concentrations of OPA.[15] The range of the strips was from 0 to 200 ppm OPA. The OPA test strips, color chart, and procedure are shown in Figure 4.[15] The variability in interpretation of the strips due to lighting and other factors could be improved by using a hand held colorimeter.

### 8. *Removal*

Requirements for OPA removal from IATCS coolant included the capacity to remove 95%±5% of the determined OPA concentration, without altering coolant alkalinity, no significant addition of leachate ions from the removal bed including chloride and other halogens, no contribution of particulates, and no contribution of assimilable organic carbon. Ambersorb 572® was selected because it had twice the capacity for OPA than activated carbon and had the capacity to remove 95% of OPA degradation products.[15] The resin had significantly less fines than activated carbon. The removal resin has not been used on orbit to reduce the coolant concentration of OPA or 2-FBA.



OPA Test Strip Color Chart
Polyvinyl Plastic Test Strip

Test Procedure
1. Allow test strips to remain in fluid sample for 7 min.
2. Remove test strip and allow to dry for 1 min.
3. Read and compare test pad to color chart to determine OPA concentration.

| ● | ● | ● | ● | ● |
|---|---|---|---|---|
| 0ppm | 25ppm | 50ppm | 100ppm | 200ppm |

OPA Concentration

Notes

| [OPA] (ppm) | PANTONE Formula Code |
|---|---|
| 0 | 223c |
| 25 | 514c |
| 50 | 522c |
| 100 | 666c |
| 200 | 430c |

**Figure 4. OPA Test Strips, Color Chart, and Procedure[15]**

*9. Performance History*

OPA has effectively inhibited the growth and recovery of viable microorganisms in the IATCS coolant when the concentration of OPA was maintained at greater than 50 ppm. Re-growth of bacteria in the U. S. Lab occurred within a month after initial addition of 100 ppm when the concentration of OPA dropped below the inhibitory concentration due to reaction with microorganisms and surface passivation. Standard practice for antimicrobial implementation usually recommends the addition of 5 to 10 times the minimum effective dose to kill the most insensitive bacteria in diffusion limited areas and prevent growth of resistant organisms.[16] OPA coolant concentration was increased to reduce the risk of development of OPA resistance and reduce ground to orbit transport logistics after Shuttle retirement. The OPA “as circulated in flight hardware” allowable concentration of OPA in IATCS coolant was increased to 25 – 500 ppm in document SSP 30573 Revision E, Table 4.1-2.8 Heat Transport Fluid (IATC). OPA degradation rate in most modules has slowed to less than 0.3 ppm/day. The on-orbit addition and maintenance of OPA in ISS elements is shown in Figure 5. The comprehensive systematic methodology for testing and implementation of a new antimicrobial in the ISS IATCS is a best practice for future antimicrobial implementation in spacecraft water systems.

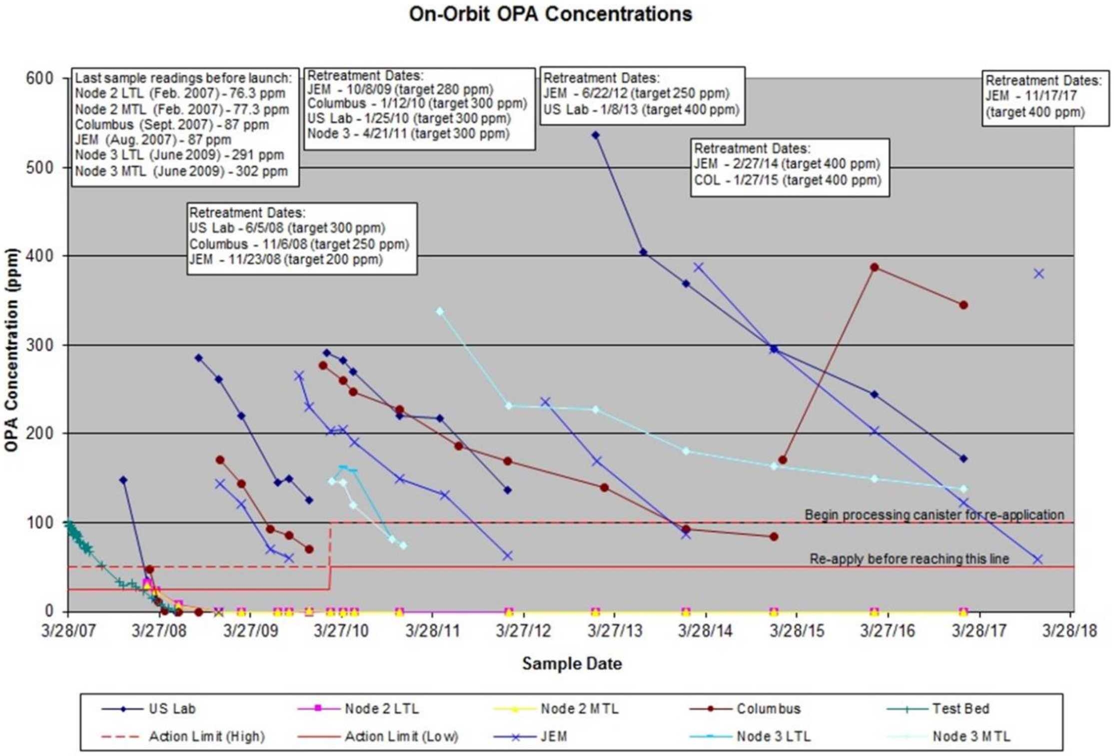


**Figure 5. OPA Addition and Maintenance in ISS Elements.**

## D. EMU Water Loop Iodine Antimicrobial

The EMU Feed-water loop provides water to a Sublimator porous plate for system cooling. Heat is rejected by the sublimation of the Feed-water water to the vacuum of space. The Feed-water tank provides roughly 3.8 kg of water for cooling along with storing crew respiration and perspiration condensate from the ventilation loop. The Cooling Water Loop transfers the crew heat load to a Sublimator for cooling. Crew thermal comfort is manually controlled by varying the Cooling Water Loop flow to the Sublimator. Iodine, at 1 - 4 ppm concentrations, is the antimicrobial used in both of these water loops since the inception of the EMU Program.

The requirement for the operational life of the EMU hardware evolved from 7 – 10 days on-orbit during the Shuttle era, to up to 6 years on-orbit for the ISS Program. Maintaining the EMU Cooling Water Loop for long-term (6 years) operation resulted in significant challenges. The Cooling Water Loop Fan/Pump/Separator and key Cooling Water Loop filters have failed in the past due to chemical and microbial contaminants after long-term static storage with no maintenance of the water quality.

In 2003 for instance, three EMU systems (serial numbers 3005, 3011 & 3013) were left static and not in use on-board the ISS after the Columbia accident and began to experience significant performance degradation and failure within approximately a year. The EMU Cooling Water Loop fan/pump/separators did not function due to a build-up of inorganic scale and organic material (polysaccharides), most of which was identified as biofilm noted by arrows in Figure 6.

The Airlock Cooling Loop Recovery (ALCLR) water processing kit was developed as a corrective action to the EMU Cooling Water Loop flow disruptions experienced on the ISS due to long-term static storage. The components in the kit are designed to remove the contaminants that caused prior flow disruptions. ALCLR water processing kits have been utilized since 2004 as standard operating procedure. Periodic analysis of EMU Cooling Water Loop water and hardware examinations have been used as a means to determine adequate functionality, optimized processing cycles, and ALCLR component shelf-life. Since its implementation, the ALCLR processing has done a good job of maintaining water quality, dropping planktonic microbial counts in the water from the $10^7$ CFU/100 mL to $10^4$ CFU/100 mL or less (primarily *Pseudomonas* subspecies).

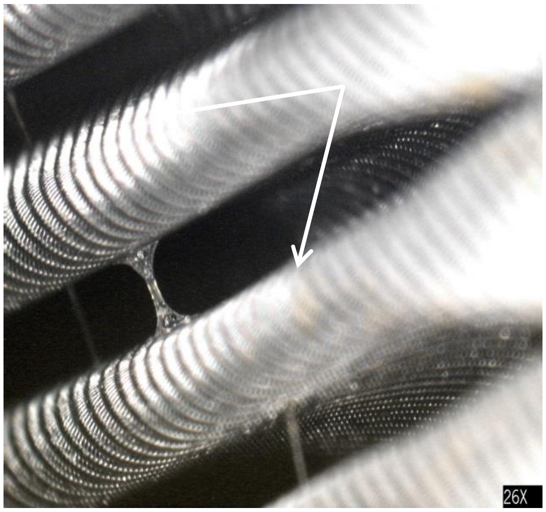


**Figure 6. Biofilm on EMU Cooling Water Loop Gas Trap**

The ALCLR water processing kit (Figure 7) was devised to scrub and remediate the various chemical and biological contaminants and byproducts that were found to have fouled the magnetically coupled pump in the EMU Cooling Water Loop Fan/Pump/Separator. The heart of the kit is the EMU Ion Filter, which initially was a 50:50 by volume packed bed of mixed anion/cation exchange resin and activated carbon. EMU Ion Filter is periodically installed into the EMU and Airlock Heat Exchanger coolant loop and serves the purpose of removing inorganic and organic constituents, such as nickel and iron corrosion products, and organic acids with the ion exchange resin. Furthermore, uncharged organic contaminants are removed with the activated carbon. Finally, a Biocide Filter that imparts 1 – 4 ppm iodine antimicrobial into the cooling loop water is utilized for disinfection of the EMU Cooling Water Loop after the cleaning process. This process occurs once every 90 days when the EMU hardware is not being used, and before and after each series of Extravehicular Activity (EVA) when the EMU hardware is in use.[8]

A recent re-design of the ALCLR test kit to integrate an in-line conductivity sensor has been undertaken. The implementation of a simple off-line means to determine EMU coolant water pH, has been integrated as well. Also, a simplified means to acquire on-orbit EMU cooling water samples has been part of the re-design effort. Finally, an inherently cleaner organic adsorbent, to replace the current lignite-based activated carbon, and a non-separable replacement, for the separable mixed ion exchange resin, have been selected for the design. These efforts were undertaken to enhance the performance and reduce the risk associated with ALCLR operations to ensure the long-term health of the EMU cooling water circuit.[8]

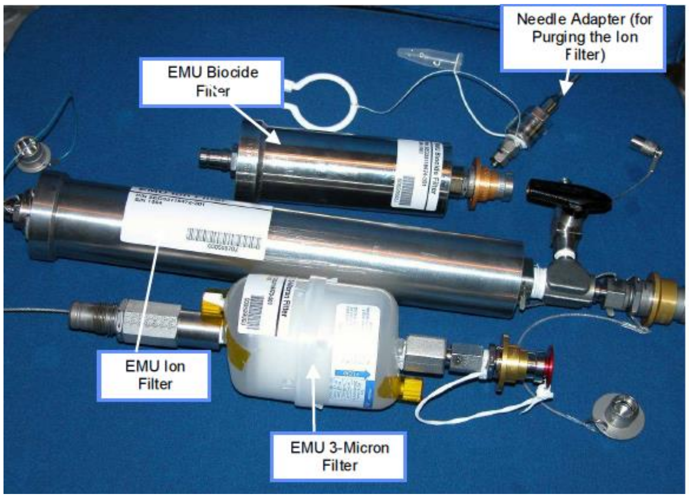


**Figure 7. EMU ALCLR Water Processing Kit.**

The EMU Feed-water bladder reservoirs are loaded with 1 – 4 ppm iodine as an antimicrobial as well. If that water is not used for an EVA, it is discharged and recharged with fresh water containing 1 – 4 ppm iodine once every 6 months. Though there has been no off-nominal performance issues associated with microbial activity in the EMU Feed-water loop, periodic disinfection was judged to be a prudent thing to do.

### E. Common Cabin Air Assembly (CCAA) Condensing Heat Exchanger (CHX) Antimicrobial Hydrophilic Coating

The ISS CCAA CHX condensing surfaces and slurper bars utilize a hydrophilic coating to facilitate the collection of humidity condensate in a microgravity environment (Figures 8 and 9). Condensed water would pool within the slurper bars if this coating were not present. The inorganic coating is a silica/silicate-based, sol-gel-type which contains a silver salt as the antimicrobial agent to meet the demanding, long-term antimicrobial requirements of the ISS condensing heat exchangers. Antimicrobial protection in this hardware is important for several reasons.[9]

**Figure 8. Condensing Heat Exchanger**

**Figure 9. Antimicrobial Hydrophilic Coating on Test Panels**

In operation, the condensing surfaces of the CHX are wet for extended periods of time. Furthermore, volatile and semi-volatile organic compounds are known to collect on these surfaces. Since the surfaces are not sterile, and microorganisms can be introduced to these surfaces, there would be a high risk of microbial proliferation if active microbial control were not implemented. Microbial proliferation can lead to biofilm formation such that hardware functionality could be jeopardized via fouling of convoluted CHX fin-stock and/or slurper bars that are used to collect humidity condensate for water recycling. Furthermore, unchecked microbial activity within the CHXs can lead to crew-cabin air passing through the hardware and becoming contaminated with microorganisms, leading to unhealthful air to the crew or odor generation. Finally, unchecked microbial activity within the CHXs can lead to microbiologically influenced corrosion.

The antimicrobial hydrophilic coating used on the condensing surfaces of the ISS has demonstrated long-term antimicrobial character in ground testing (up to 3 years – Figure 10) and for hardware returned to the ground after 6 years of flight operation where no viable microorganisms were isolated from the condensing surfaces. The long-term antimicrobial character is due to the fact that the solubility of the hydrophilic coating ingredients are well-matched. That is, low dissolution of the hydrophilic coating occurs over time as humidity condensate passes through it, and silver salt is equally dispersed throughout it. The coating is used in concert with a periodic dry cycle on the ISS to maximize control of fungal activity. Testing indicated that no periodic dry cycle was necessary for bacterial control.[9]

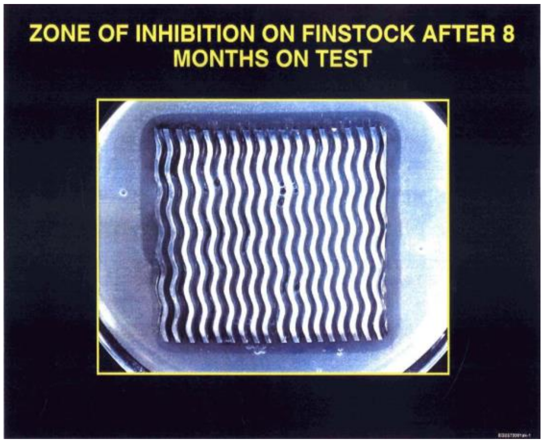


**Figure 10. Zone of Inhibition Test after 8 Months of Simulated System Use**

## V. Orion Era Antimicrobials

The first manned flight of Orion is the Exploration Mission 2 (EM-2). EM-2 has two water systems that require water quality maintenance, the Potable Water System (PWS) and the Liquid Cooling Garment Water Cooling System (LWC).

### A. PWS Antimicrobial Overview

The PWS resides in the European Service Module and provides potable water to crewmembers in the Crew Module. The PWS utilizes a nominal dose of 400 ppb silver fluoride to control microbial activity. Historically, molecular iodine has been used for microbial control of potable water in space systems. Molecular iodine is effective in preventing microbial activity, but the iodine must be removed with ancillary hardware, prior to human consumption, due to potential thyroid implications, in addition to taste and odor issues. Ionic silver ($Ag^+$) provides more convenience and less hardware mass implications – as certain levels of $Ag^+$ are deemed safe for human consumption. For Orion, 400 ppb was selected as the ionic silver biocide dosage as it is the maximum consumable limit as defined by the NASA Human Systems Integration Requirements Document (MPCV 70024). The use of silver fluoride biocide maintains potability of the PWS water, while limiting microbial growth to less than 50 CFU/mL at point of consumption – a limit also set by the NASA Human Systems Integration Requirements Document (MPCV 70024). The PWS is different from previous NASA potable water systems in the fact that it is not re-dosed or monitored during post-loading. Therefore, the antimicrobial must remain effective for periods upwards of 300-350 days, the expected length of time between vehicle PWS processing and mission completion.

Materials implications for PWS antimicrobial selection proved to be challenging. While $Ag^+$ provides some advantages in regards to human potability, it is also known to lose efficacy due to silver plating on the wetted system surfaces. The PWS consists of 4 Titanium 6Al-4V water tanks which contain stainless steel 316L bellows. The potable water is in contact with both the Titanium 6Al-4V shell and stainless steel 316L bellows. The water tubing in the Potable Water System is stainless steel 316L and the water tubing in the Crew Module is Titanium 6Al-4V. During extensive materials testing, scientists at NASA JSC defined an appropriate cleaning and pre-treatment program that controls particulate generation, and maintains antimicrobial concentrations at desired levels for the necessary timelines associated with the vehicle operations and missions. Based on this information, the current concept of operations for loading of the EM-2 PWS begins in the Vehicle Assembly Building up to 280 days before launch. The EM-2 loading and sampling concept of operations has not been finalized at this point, but is expected to generally follow the plan outlined here. The system should be initially cleaned to 200A levels (limits 200 µm particles to 1.0/L, 100 µm particles to 160/L, 50 µm particles to 1700/L, 25 µm particles to 12,400/L, 15 µm particles to 41890/L and non-volatile residue to 10 mg/L as defined in MIL-STD-1246C). The water tanks are initially loaded with a disinfection load of deionized water with 500 ppm silver fluoride. This solution will sit in the water tanks for 24 hours in an effort to provide disinfection and some passivation of the system. After 24 hours, the disinfection solution is drained from the tank. The system is then refilled with the flight solution, deionized water with 400 ppb silver fluoride. After dwelling for 1 hour, the system is purged to remove all of the 500 ppm silver fluoride solution from the bellows. The water tanks are then refilled a final time with the same solution of deionized water with 400 ppb silver fluoride. After this flight load, the system fluid is sampled for biological and chemical components. If the system is compliant with Orion Multi-Purpose Crew Vehicle Program Human-Systems Integration Requirements (HSIR) (MPCV 70024), the system will be closed and considered ready for launch.

### B. Liquid Cooling Garment Water Cooling System (LWC)

The second Orion system that utilizes an antimicrobial is the LWC, located in the Crew Module. This is a closed loop, water-based system that provides crewmember cooling via recirculating cold water through the liquid cooling garment (LCG). Water quality must be maintained in this system in order to ensure system degassing through the passive membrane gas trap, as well as to maintain loop delta pressures. In order to streamline vehicle processing, and also to maintain future capability, NASA baselined the same antimicrobial and nominal dose for the LWC as used in the PWS – 400 ppb silver fluoride. However, due to materials concerns, the LWC will not undergo the higher 500 ppm disinfection load utilized by the PWS.

The crewmembers wear the LCG while suited for launch and re-entry. The LWC system consists of a liquid-liquid heat exchanger that interfaces with the external coolant loop, a pump, a water accumulator, a gas trap, the umbilical interface, and it interfaces with the 4 LCGs and their associated hardware. The main metallic materials for the vehicle-side of the LWC consists of Titanium 6Al-4V and Titanium 3Al-2.5V (pump, tubes, fittings), various 300-series passivated stainless steels (fittings, restrictors, heat exchanger), Inconel 718 (bellows in tank), and Ebrite (valve surfaces). Unlike the PWS, in the LWC water tank, water is in contact with Inconel 718 bellows only. The water does not contact the tank shell. The LWC gas trap consists of polypropylene and polyethylene. The LCG system, which interfaces with the LWC, includes polyvinyl chloride and polytetrafluoroethylene tubing and aluminum and titanium valve materials.

The final concept of operations for loading the LWC has not been finalized, but is expected to include loading the LWC with 400 ppb silver fluoride in deionized water solution approximately 840 - 1240 days before launch. The water will be circulated and sampled. If the water meets the requirements as outlined for potable water in the HSIR (MPCV 70024), the system will be considered ready for launch. Based on future materials testing to analyze the effects of LWC loop materials on antimicrobial concentrations and efficacy, a sampling, scrubbing and re-dosing schedule will be defined in order to maintain water quality in the system.

## VI. Exploration Era Antimicrobials

The United States has used iodine successfully on the ISS for water disinfection. Iodine imparts a bad taste and its use requires removal of the iodine before consumption, due to its adverse effects on the thyroid. Switching to silver for extended exploration missions is essential because silver is relatively harmless to the body at bactericidal levels and 99% of silver is excreted by the body.[17] Therefore, no expendable adsorbents are required for use.

Many factors are known to interfere with the antimicrobial activity of $Ag^+$. These include temperature, pH, phosphates, chlorides, calcium, sulfides, organics, and colloidal particles.[13] Silver ions also readily react with system materials and must be maintained at lethal concentrations >50 ppb to reduce the risk of the development of antimicrobial resistance.

Concepts for a membraneless silver ionization reactor consisting of tubular, concentric silver electrodes to avoid stagnant flow within the reactor to produce greater than 1000 ppb $Ag^+$ concentration have been developed and tested.[17] Reversing the polarity of the electrode avoided dendritic silver growth that can short circuit the cell. And a mechanical cleaning mechanism was developed to periodically drag an internal cleaning rod, placed between the electrodes to remove the oxide film layer that builds up.[17] A starting concentration of greater than1000 ppm $Ag^+$ should maintain lethal concentrations of silver throughout the water storage and distribution system to prevent the growth of pathogenic bacteria and suppress biofilm formation.

Another option for the use of silver to disinfect water for extended duration with a shelf life greater than five years is a silver ion complex called silver dihydrogen citrate (SDC). As described by Pure Bioscience at https://www.purebio.com/technology/silver-dihydrogen-citrate-sdc.htm, the unique bond of the silver ion allows the silver to remain in solution while making it more bio-available for antimicrobial action. SDC kills microorganisms by two modes of action, including deactivation of structural and metabolic membrane proteins, and as a food source allowing the silver to enter the microorganisms and readily denature deoxyribonucleic acid (DNA) in the cytoplasm. The citrate serves as a “Trojan horse” for silver ions making SDC highly and quickly effective against a broad spectrum of microorganisms. No resistance to SDC has been identified to date. The recommended dose range for water disinfection is 500 ppb to 10 ppm. The minimum effective concentration for water disinfection is 78 ppb. SDC is relatively non-toxic, but chlorides can adversely impact the stability and effectiveness.

One additional aspect of disinfection is the concept of remediation after a contamination event during long-duration exploration missions far away from Earth interactions. In many cases, such as biofilm formation or the development of disinfectant resistance in the resident microorganisms, contamination may create unforeseen performance problems in spacecraft systems. In the case of disinfectant resistant microorganisms, the addition of higher concentrations of disinfectant may not be effective. Future research and novel engineering approaches are needed to mitigate the impact of such events during these missions.

## VII. Summary

The use of antimicrobials to control microbiological growth in manned spaceflight water-based systems has and will continue to have a unique set of challenges and needs. The challenges are varied, and include antimicrobial effectiveness, crew health and safety, materials compatibility, optimal system functionality, antimicrobial shelf life, means to monitor antimicrobial concentration and means to re-introduce biocides periodically in the case of depletion. Needs vary from application to application, and include control of pathogens for crew health, control of biofilm formation for optimal system functionality, inhibition and prevention of microbiologically influenced corrosion, optimization of wetted metallic material life, and general living quarter and consumable aesthetics with respect to odor and taste. Antimicrobials should be added at a dose sufficient to kill the most insensitive microorganisms in diffusion limited areas to reduce the risk of development of antimicrobial resistance. Antimicrobials should also be maintained at concentrations greater than the minimum inhibitory concentration after initial degradation due to surface deposition.

The intent of this paper was to outline and discuss the various antimicrobials used in prior and current manned spaceflight water-based applications with focus on pros, cons, and lessons learned. There was no intent to pick one optimal antimicrobial for all applications, and no such antimicrobial is likely to exist. Design factors such as minimum inhibitory concentration, minimum lethal concentration, required circulated concentrations, materials selection, means to introduce, means to monitor real-time, and concentration maintenance are all key factors to be taken into account when selecting an antimicrobial for any application. The challenges associated with longer term missions as well as long-term system dormancy as envisioned for exploration missions, lunar habitats and a manned Mars mission are expected to result in more complex challenges with respect to anticipated needs and potential design solutions for the antimicrobials to be selected.

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