

International Space Station (ISS) Internal Active Thermal Control System (IATCS) New Biocide Selection, Qualification and Implementation

Mark E. Wilson¹

The Boeing Company, Houston, TX, 77059

Harold E. Cole²

The Boeing Company, Huntsville, AL, 35824

Tony Rector³, John Steele⁴, and Jerry Varsik⁵

Hamilton Sundstrand Space Systems International, Inc., Windsor Locks, CT, 06095

The Internal Active Thermal Control System (IATCS) aboard the International Space Station (ISS) is primarily responsible for the removal of heat loads from payload and system racks. The IATCS is a water based system which works in conjunction with the EATCS (External ATCS), an ammonia based system, which are interfaced through a heat exchanger to facilitate heat transfer. On-orbit issues associated with the aqueous coolant chemistry began to occur with unexpected increases in CO₂ levels in the cabin. This caused an increase in total inorganic carbon (TIC), a reduction in coolant pH, increased corrosion, and precipitation of nickel phosphate. These chemical changes were also accompanied by the growth of heterotrophic bacteria that increased risk to the system and could potentially impact crew health and safety. Studies were conducted to select a biocide to control microbial growth in the system based on requirements for disinfection at low chemical concentration (effectiveness), solubility and 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. Based on these requirements, ortho-phthalaldehyde (OPA), an aromatic dialdehyde compound, was selected for qualification testing. This paper presents the OPA qualification test results, development of hardware and methodology to safely apply OPA to the system, development of a means to remove OPA, development of a rapid colorimetric test for measurement of OPA, and the OPA on-orbit performance for controlling the growth of microorganisms in the ISS IATCS since November 3, 2007.

Nomenclature

<i>BMP</i>	=	Russian system for removal of organic contaminants from air
<i>CCAA</i>	=	common cabin air assembly
<i>CFU</i>	=	colony forming units
<i>CPP</i>	=	cyclic potentiodynamic polarization

¹ Associate Technical Fellow, Boeing Research & Technology, 13100 Space Center Blvd., MC HB3-20, Houston, TX 77059.

² Associate Technical Fellow, Boeing Research & Technology, 499 Boeing Blvd., MC JW-66, Huntsville, AL 35824.

³ Senior Engineer, Engineering Specialists, Hamilton Sundstrand Space Systems, 1 Hamilton Road, MS 1A-2-W66, Windsor Locks, CT 06098-1010.

⁴ Technical Fellow, Engineering Specialists, Hamilton Sundstrand Space Systems, 1 Hamilton Road, MS 1A-2-W66, Windsor Locks, CT 06098-1010.

⁵ Manager, Engineering Specialists, Hamilton Sundstrand Space Systems, 1 Hamilton Road, MS 1A-2-W66, Windsor Locks, CT 06098-1010.

<i>EATCS</i>	= external active thermal control system
<i>ECLSS</i>	= environmental control and life support system
<i>EPR</i>	= ethylene propylene rubber
<i>IATCS</i>	= internal active thermal control system
<i>ISS</i>	= International Space Station
<i>JEM</i>	= Japanese Experiment Module
<i>L</i>	= liter
<i>MIC</i>	= minimum inhibitory concentration
<i>ml</i>	= milliliters
<i>MLC</i>	= minimum lethal concentration
<i>MPY</i>	= mils per year
<i>NiRA</i>	= nickel removal assembly
<i>OPA</i>	= ortho-phthalaldehyde
<i>PhosRA</i>	= phosphate removal assembly
<i>PPM</i>	= parts per million or mg/L
<i>SKV</i>	= Russian system for collection of humidity condensate
<i>TCCS</i>	= trace contaminant control system
<i>TIC</i>	= total inorganic carbon
<i>USOS</i>	= United States On-orbit Segment

I. Introduction

SINCE 2001, chemical and microbiological analyses of the IATCS coolant has been performed on grab samples returned to the ground. Stegman et al. described the mechanisms responsible for the decrease in pH to 8.4 from the specification of pH 9.5 +/- 0.5 including diffusion of CO₂ into the coolant combined with insufficient alkalinity from the borate buffer to keep the pH greater than 9, an increase in nickel concentrations in the coolant, and a decrease in phosphate.^{1,2,3} A silver salt biocide, silver sulfate, initially used as an additive in the coolant formulation at a concentration of 0.1 – 3 parts per million (ppm) to control growth and proliferation of microorganisms rapidly decreased below detection limits within a few hours. Many factors can interfere with the antimicrobial activity of silver ions. These include temperature, pH, phosphates, chlorides, calcium, sulfides, organics, and colloidal particles.⁴ Over the pH range of IATCS coolant from pH 8.25 to pH 10, silver ions rapidly undergo an oxidation reduction reaction with nickel. Nickel is the reducing agent contributing electrons to silver to form silver metal and the reaction also increases the aqueous concentration of nickel ions. Repeated additions of the silver salt created a short duration increase in corrosion rates during the silver deposition process⁵. No increases in long term corrosion rates or localized pitting were noted in 180 day long term exposure testing.⁵ The rapid reduction of silver ions in the coolant coupled with decreases in pH created a favorable environment for the growth and proliferation of microorganisms. Counts of heterotrophic bacteria increased from <10 colony forming units (CFU)/100 milliliters (ml) to greater than 1.0E+06 CFU/100 ml.

Uncontrolled microbiological growth in the IATCS can deteriorate the performance of the system and potentially impact crew health and safety if opportunistic pathogens become established in the system. Microorganisms are capable of degrading the coolant chemistry, attaching to surfaces and forming biofilm, subsequent biofouling of filters, tubing, and pumps, decreasing flow rates, reducing heat transfer, initiation and acceleration of corrosion, and enhanced mineral scale formation.^{6,7,8} The majority of bacteria in nutrient limited environments such as the IATCS are attached to surfaces⁹. The ratio of planktonic (circulating) bacteria to biofilm (attached) bacteria is a function of surface energetics, materials of construction, topography, hydraulic factors, and biofilm chemistry.⁹ Disinfection to control microbiological growth and biofilm formation is necessary to maintain tolerable risk levels for the ISS IATCS due to the required operational life of the ISS IATCS for over 15 years without the ability to periodically flush and replace coolant, sterilize the system, and/or replace system equipment.

II. Biocide Development and Qualification

Boeing and NASA initiated an effort to find and qualify a replacement biocide for silver in the IATCS. The objective was the development, testing, and implementation of a stable, affordable, broad-spectrum ISS IATCS biocide for prevention of growth of circulating bacteria and formation and proliferation of biofilms, subsequent hardware fouling that may reduce flow rates and heat transfer efficiencies, and microbiological influenced corrosion.

Requirements for the replacement biocide included inhibition and death of bacteria at low chemical concentrations (effectiveness), solubility and 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.

Trade studies and rapid screening assays were used to develop a prioritized list of acceptable antimicrobial agents including glutaraldehyde, ortho-phthalaldehyde (OPA), and methyl isothiazolone.^{9,10,11,12} Glutaraldehyde at greater than 25 ppm was eliminated due to NASA concerns with safety and toxicity and methyl isothiazolone was eliminated from further consideration due to its ineffectiveness against biofilms and toxicity at higher concentrations. Therefore, OPA, an aromatic dialdehyde compound with the formula $C_6H_4(CHO)_2$ was selected for qualification testing for use as a biocide in the IATCS.

A. OPA Effectiveness Testing

A series of tests including minimum inhibitory concentrations and minimum lethal concentrations for planktonic microorganisms at pH 9.0 and 9.5, effectiveness of OPA against mixed species biofilms at pH 9.0, beaker tests for oxidative degradation of OPA, and effectiveness and stability of OPA in circulating bench and systems tests were performed to determine the effectiveness of OPA against selected microorganisms.

Minimum inhibitory concentration (MIC) testing determined the lowest concentration of a biocide that will inhibit visible growth of the test organisms⁴. The minimum lethal concentration (MLC) determined the smallest concentration of biocide that, on subculture, failed to show growth.⁴

An adaptable, rapid, sensitive, and semi-automated MIC assay was developed to screen the effectiveness of large numbers of biocides for use in aerospace applications. Labor estimates for variations of the standard MIC broth dilution tube technique for screening effectiveness of large numbers of antimicrobials exceeded available resources. Therefore, Biolog's MT2 MicroPlate™, a pre-filled microtiter plate containing tetrazolium violet (a redox dye) and minimal salts without a carbon source was selected as the test platform.¹³ The oxidation of an added carbon source by the cellular respiration of microorganisms caused a reduction of tetrazolium and a resulting purple color that was automatically recorded using the MicroStation™ plate reader. Equivalent of 8 species of gram negative non-fermenting bacteria isolated from the on-orbit IATCS and ground systems were suspended in sterile borate coolant at a concentration of approximately $1.0E+07$ cells/ml. Peptone at a concentration of 250 ppm was utilized as the carbon source except for testing aldehyde biocides which reacted with amino groups in the peptone. Aldehydes were tested using a nutrient mixture consisting of 250 ppm glucose, 250 ppm fructose, 100 ppm nicotinic acid, 100 ppm lactic acid, 300 ppm sodium pyruvate, and 25 ppm magnesium sulfate. Positive (no antimicrobial) and negative (no microorganisms) controls and 5 antimicrobial dilutions were run on each microtiter plate. Results were determined based on absorbance readings after 24 to 48 hours of incubation with <45 units indicating no growth (no purple in well), 45-90 units as slight growth (very light purple in well), and greater than 90 units as active growth (purple wells).¹³

1. OPA Effectiveness at pH 9.0

OPA at pH 9.0 showed effective microbiological control with a MIC of 10 ppm, MLC of 15 ppm, and complete kill of mixed species biofilms on BNi2 and BNi3 in less than 24 hours.

2. OPA Effectiveness at pH 9.5

Testing at pH 9.5 with microorganisms isolated from biofilms on hardware returned to the ground resulted in a MIC of 5 ppm (no significant difference from tests at pH 9.0 of 10 ppm) and a significant increase in the minimum lethal concentration to 30 ppm for all organisms tested except *Methylobacterium extorquens*. The results indicated decreased lethality of OPA to microorganisms at pH 9.5. The minimum lethal concentration of OPA for planktonic *Methylobacterium extorquens* was greater than 150 ppm.

After the determination that some microorganisms were not killed by 100 ppm OPA, testing was performed to determine the effectiveness of 400 ppm OPA at pH 9.5 against mixed biofilms consisting of 10 bacteria including *Methylobacterium extorquens* established on BNi2 and BNi3 coupons in an annular flow reactor for 38 days. A concentration of 400 ppm OPA reduced coolant concentrations of bacteria from $8.5E+07$ CFU/100 ml to less than the detection limit (20 CFU/100 ml) in 24 hours and decreased viable bacteria in biofilms from $5.0E+05$ CFU/cm² to <1 CFU/cm² in 24 hours. OPA was removed from the reactor and there was no re-growth of microorganisms within 13 days indicating high level disinfection.

3. Potential Impacts of Microgravity on OPA Effectiveness

OPA effectiveness ground test data indicated inhibition of microorganisms at concentrations of 100 ppm OPA and inhibition and death of microorganisms at concentrations of 200 – 400 ppm OPA. However, the OPA concentration required to kill space grown bacteria may be greater than 400 ppm. Nickerson et al. in an article titled “Microbial Responses to Microgravity and Other Low-Shear Environments” summarized a number of on-orbit studies confirming that space flight has a profound effect on a variety of microbial parameters, including changes in microbial growth, morphology, metabolism, genetic transfer, and viral reactivation.¹⁴ Repeated experiments have shown *Staphylococcus aureus*, *Escherichia coli*, and other microgravity grown bacteria have increased resistance to antibiotics.¹⁴ Although the mechanism for the increased resistance to antibiotics in-flight is unclear, the difference has been speculated to be the changes in microbial growth kinetics or a modification of cellular transport mechanisms.¹⁵ Dr. James Jorgensen, University of Texas Health Sciences Center, has proposed that the presence of thicker cell walls in microgravity grown bacteria makes it more difficult for biocides to penetrate the microbe, thus contributing to the loss of biocide sensitivity.¹⁵

B. Stability of OPA in the IATCS

OPA’s primary mechanism for biocidal activity is the reaction with primary amino groups found within multiple bacterial structures including proteins of the cell wall as shown in Figure 1.¹⁶ According to Gerald E. McDonnell in *Antisepsis, Disinfection, and Sterilization: Types, Action, and Resistance*, OPA has been shown to be more penetrating than glutaraldehyde and to be more effective on proteins within bacterial and fungal cell walls and membranes.¹⁷ “This may be due to differences in the structure of OPA under the hydrophilic conditions observed at the external surface of the cell; it adopts a locked structure with unexposed aldehyde groups and allows penetration of the biocide into the cell. Once within a hydrophobic environment, typical of the cell wall or membrane, it is proposed to assume a more open, exposed form with reactive aldehyde groups.”¹⁷ OPA’s ability to undergo covalent hydration in an aqueous environment is shown in Figure 2.¹⁶ The equilibrium is actually shifted toward the hydrated form because of the electron-withdrawing and “ortho-effect” of the second carbonyl group. An intramolecular nucleophilic attack of an oxygen atom of the CH(OH)₂ group on the remaining carbonyl group occurs, resulting in a cyclic hemiacetal.¹⁶

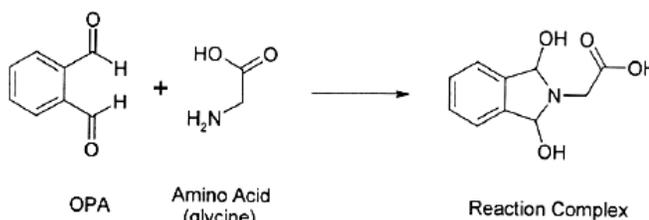


Figure 1. Reaction of OPA with a primary amino acid (Glycine)¹⁶.

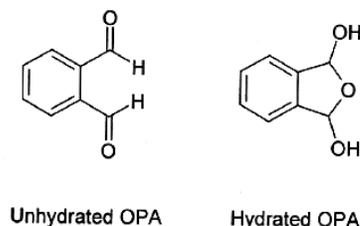


Figure 2. Comparison of the un-hydrated OPA Structure with the Hydrated Cyclic Hemiacetal¹⁶.

Because the OPA molecule lacks an alpha-hydrogen, the Aldol condensation polymerization reaction that occurs with glutaraldehyde at alkaline pH does not occur.¹⁶ Therefore, OPA is stable over a wider pH range from acidic (pH 3) to alkaline (pH 9). OPA can undergo a reduction-oxidation intramolecular Cannizzaro reaction, but this process usually only occurs in alkaline solutions with pH > 10 and elevated temperatures.¹⁶ Initial testing to identify OPA degradation products in grab samples returned from the ISS indicated a complex mixture of oxidation products including benzenedicarboxylic acids, alcohols and reaction products of OPA and ammonia.

OPA degradation rate in the Systems Test was approximately 0.26 ppm/day as shown in Figure 3. Coolant from the systems Test and

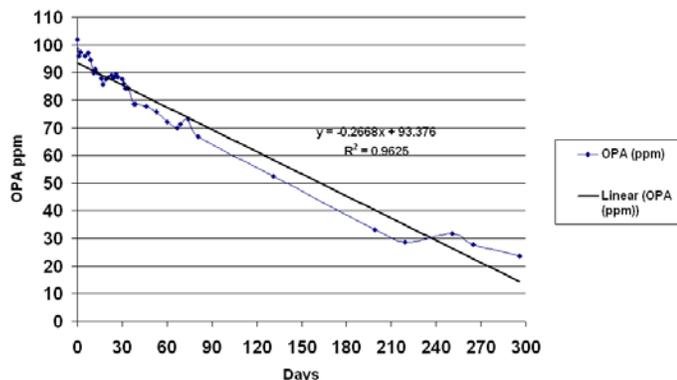


Figure 3. OPA degradation in the systems test

the US Laboratory ITCS were analyzed at Microbac Laboratory in North Carolina using triple quadrupole LC/MS/MS systems. There were 3 modes of degradation observed in the samples including oxidation reactions from base/metal catalysis, base catalyzed reactions, and reaction with ammonia. The major degradation product formed is 2-formylbenzoic acid (2-FBA), likely due to catalytic oxidation with Nickel 201 and Nickel braze alloys in the IATCS. Figure 4 lists 2-formylbenzoic acid as Compound 1. The alkaline pH of the system may also be contributing to the degradation rate of OPA. There is evidence that the 2-FBA is further oxidized to phthallic acid. Ammonia has been shown to readily react with OPA to produce compounds 4 and 4'. Chemical methodologies have not been able to discern the differences in the structures of the ammonia reaction product. Compound 5 has also been detected in used ITCS fluids. Compound 5 can undergo base catalyzed hydrolysis to produce compound 6, or compound 6 can be produced from OPA through the well known Cannizzaro redox mechanism. Compounds 7 and 8 have been detected at low levels, but their origin is not clear at the present time. In summary, there are many

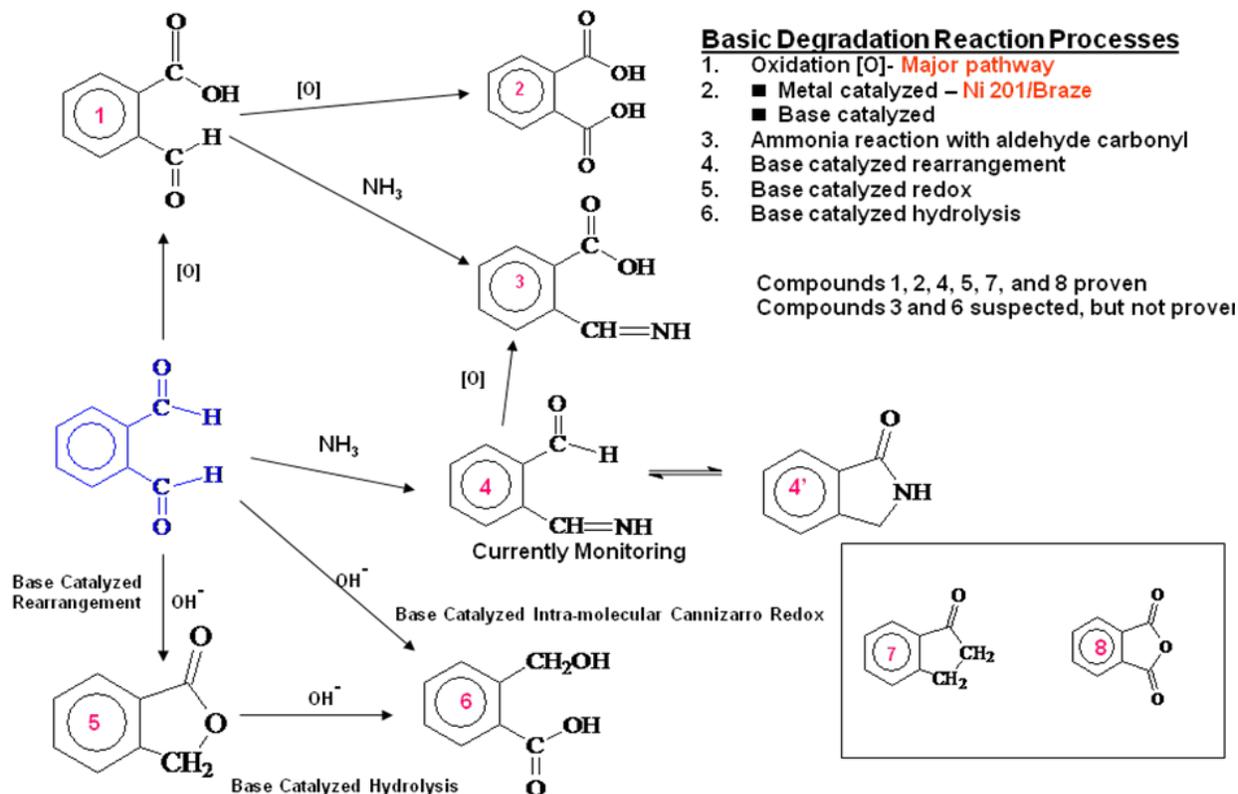


Figure 4. Proposed structures and origin of OPA reaction products.

possible reactions which contribute to the degradation of OPA, however it appears that the most prevalent degradation process involves catalytic oxidation of OPA to 2-FBA in the presence of nickel braze alloys.

C. Material Compatibility with IATCS Soft Goods and Hardware

In order to test for material compatibility, non-metallic materials including Nylon 11, Nylon 66, polypropylene, Valox® (polybutylene terephthalate), ethylene propylene rubber (EPR), epoxy highly filled casting material, and unfilled epoxy resin were exposed for 90 days in 0.04M carbonate/ bicarbonate buffer at pH 9.1 and analyzed for changes in tensile stress, elongation, weight gain, and volume swell with interval tests every 30 days. Only weight gain and volume swell were performed on epoxies exposed for 90 days with interval tests every 30 days. All of the materials tested displayed a lack of significant changes in physical and mechanical properties indicating coolant compatibility. Nylons exhibited degradation in tensile strength, weight gain, and volume swell attributable solely to the absorption of water.¹⁸

None of the nonmetallic materials exhibited significant compatibility differences following exposure to 100 ppm OPA and the coolant/biocide combinations previously evaluated. Materials (polypropylene, Valox, EPR, and filled and unfilled epoxy) that were determined to be compatible with previously considered coolant/biocide combinations have also demonstrated compatibility with OPA. As determined previously, observed changes to material properties appear to be due to the primary coolant, independent of the biocides under investigation. Those materials

demonstrating sensitivity to aqueous solutions (Nylon 11 and Nylon 66) exhibit similar performance when exposed to OPA solutions.¹⁸

Ninety day exposure testing was performed on metallic materials in 0.04 M carbonate/bicarbonate buffer at pH 9.1: 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. Metallurgical cross sections and scanning electron microscope surface examinations for all materials indicated corrosion rates less than observed for IATCS coolant at pH 8.4. Additional stress corrosion cracking testing of the CRES 15-5PH and 17-7PH materials found no evidence of stress corrosion cracking.¹⁹ At 100 ppm OPA, all the metallics exhibited a Cyclic Potentiodynamic Polarization (CPP) average corrosion rate of < 0.01 mils per year (mpy) except for Nioro repair which had an average corrosion rate of 0.02 mpy.¹⁹ At 200 ppm OPA, all the metallics exhibited a CPP calculated average corrosion rate of <0.01 mpy except for 2 materials with higher corrosion rates including the BNi-3 single braze (0.03 mpy) and the Nioro (0.04 mpy).¹⁹ However, these small increases in corrosion were determined to not impact operability and system life.

Additional studies were performed including CPP and immersion testing for determination of metal corrosion rates at 200 - 600 ppm OPA on metallic materials determined to be the most sensitive to OPA (BNi-3 braze and Nioro braze repaired BNi-3 coupons). Corrosion rates determined by CPP were approximately 2 times higher than corrosion rates determined by immersion testing.²⁰ Corrosion rates for BNi-3 were 0.05 – 0.12 mpy by CPP and < 0.06 mpy by immersion.²⁰ Corrosion rates for Nioro braze repaired BNi-3 were 0.12 mpy by CPP and 0.10 mpy by immersion.²⁰

The effect of 600 ppm OPA on non-metallic polymeric materials was determined by immersion testing for 90 days. Nylon 11 and Nylon 66 exhibited reduced water absorption with increasing OPA concentration.²¹ Polypropylene exhibited evidence of a slight increase in embrittlement, but only at a ‘threshold’ level and not indicative of incompatibility. None of the other materials tested exhibited changes from previous testing. The minor changes caused by exposure to 600 ppm were not considered to adversely impact hardware function and life.²¹

Based on the OPA material compatibility data, the recommendation was made to increase the “as circulated in flight hardware” allowable concentration of OPA in the IATCS coolant to 25 - 500 ppm (SSP 30573 Revision E, Table 4.1-2.8 Heat Transport Fluid (IATC). The higher specification for OPA was approved to reduce ground to orbit transport logistics after Shuttle retirement.

D. Toxicological Evaluation of OPA

Evaluations of the toxicity of OPA were conducted by NASA Johnson Space Center Toxicology Group and were provided in Memo 632 dated March 4, 2005, Memorandum TOX-MC-2007-TBD dated August 27, 2007, Memorandum TOX-MC-2008-11 dated June 27, 2008, and Memorandum TOX-HG-2009-09 dated April 30, 2009.^{22,23,24,25} Circulated concentrations of OPA up to 500 ppm were determined to be Toxicity Hazard Level 0 (Nonhazard) defined as slight irritation that lasts <30 minutes and will not require therapy for all toxicology parameters.^{22,24} 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 >30 minutes and will require therapy due to irritation of soft tissues such as the eye.²⁴ If coolant containing OPA leaks and the water evaporated until OPA became saturated, the resulting concentrate would be a severe eye irritant classified as a Toxicity Hazard Level 2 (Catastrophic) defined as moderate to severe irritation that has the potential for long-term performance decrement and will require therapy.²⁴ Solid OPA was determined to be a Toxicity Hazard Level 2 (Catastrophic) for eye and skin irritancy and a Toxicity Hazard Level 1 (Critical) for inhalational and systemic toxicity.²⁴ OPA in rare instances has caused allergic reactions from repeated skin contact with residual liquids or solids. OPA loaded resin beads containing 0.25 g/cm² OPA in the antimicrobial applicator was assessed as a Toxicity Hazard Level 0 (Nonhazard)²³. Further evaluation of leaked coolant containing OPA that dried as a film on surfaces was determined to be a Toxicity Hazard Level 0 for eye contact.²⁵ Wet towels and wipes may be utilized to remove the film from surfaces and are still considered Toxicity Hazard Level 0 since any OPA residue would be trapped in the matrix.²⁵

E. ECLSS Evaluation of OPA

Jay L. Perry (NASA MSFC ISS Air Quality Control Systems, ECLSS Design and Development Branch) performed an ECLSS compatibility assessment of OPA in 2005. This engineering evaluation determined that the OPA concentration in the working fluid in the IATCS of ISS (at assembly complete) should not exceed 109 mg/L.²⁶

This conclusion was based on the leakage specifications of the modules in the US On-orbit Segment (USOS), Columbus Module, and Japanese Experiment Module and scrubbing capability provided by the US trace contaminant control system (TCCS), the Russian BMP which removes organic contaminants from air in the Russian elements, and by humidity condensate absorption for a crew of three from the Common Cabin Air Assembly (CCAA) and Russian SKV. Even though OPA has a relatively low vapor pressure, its high water solubility has the greatest impact on ECLSS with impacts on multifiltration resin bed life and potential break through overwhelming the volatile removal assembly in the water processor. The maximum concentration of OPA allowed in the humidity condensate resulting from an IATCS fluid leak is 5 mg/L. A minimum OPA concentration in the ISS cabin atmosphere of 0.05 mg/m³ is required to contribute a 5mg/L OPA concentration to the humidity condensate. A combined specification leakage rate of 14.7cc/hr (LTL and MTL for all USOS modules) was used to assess the OPA cabin atmosphere concentration dynamics as a function of OPA fluid loop concentration. At leakage rates >14.7 cc/hr, an OPA concentration of 109 mg/L would result in cabin atmosphere concentration of ≥0.05mg/L and the OPA concentration in humidity condensate would be ≥5mg/L. Therefore, the OPA fluid specification was recommended to be 75 – 105 ppm.²⁶

The initial assessment was very conservative based on actual leakage rates for several modules. Another engineering analysis was performed by Ariel V. Macatangay, Ph.D., Wyle Laboratories, on March 10, 2008 based on the actual leakage rates and specification leakage rates for modules without leakage rate data.²⁷ The total leakage rate for all modules was 7.08cc/hr. This is less than half of the specification leakage rate previously used. Increasing OPA concentration in all loops would result in an OPA cabin concentration of approximately 0.05 mg/m³ at the 7 cc/hr leakage rate (25). For a concentration of 200 ppm OPA, the ISS OPA cabin concentration would be approximately 0.03mg/m³ at the same leakage rate of 7cc/hr.²⁶ This assumes leakage from all ISS modules at assembly complete, with scrubbing from the TCCS, BMP, and by humidity condensate absorption for a crew of three by the SKV and CCAA. An increase to a crew of 6 would slightly raise allowable OPA cabin concentrations by 4% due to the increased production of humidity condensate²⁷.

The maximum concentration of OPA in the IATCS coolant is highly dependent on the management of leakage scenarios. The ability for rapid leak detection, isolation, and OPA neutralization is required to reduce risk. The development of a flight rule addressing allowable IATCS leakage rates at various OPA concentrations was strongly recommended.²⁷

F. OPA Delivery

The method to deliver OPA to the IATCS coolant was an immobilization of the active biocide to a solid substrate.²⁸ The immobilization process involved a solvent evaporation technique that allowed the OPA to be physically constrained in a porous resin material. Once the resin material was exposed to the coolant fluid, the OPA effectively eluted from the resin material into the aqueous phase. This implementation was selected due to system requirements and the use of pre-existing flight hardware. System requirements included limits on the initial and final OPA concentration observed eluting from the canister (reactor) and compatibility with the coolant loop chemistry (i.e. no leachate, particles, adverse effects to chemistry/system materials).²⁸

The flight hardware used for the OPA delivery was a refurbished Nickel Removal

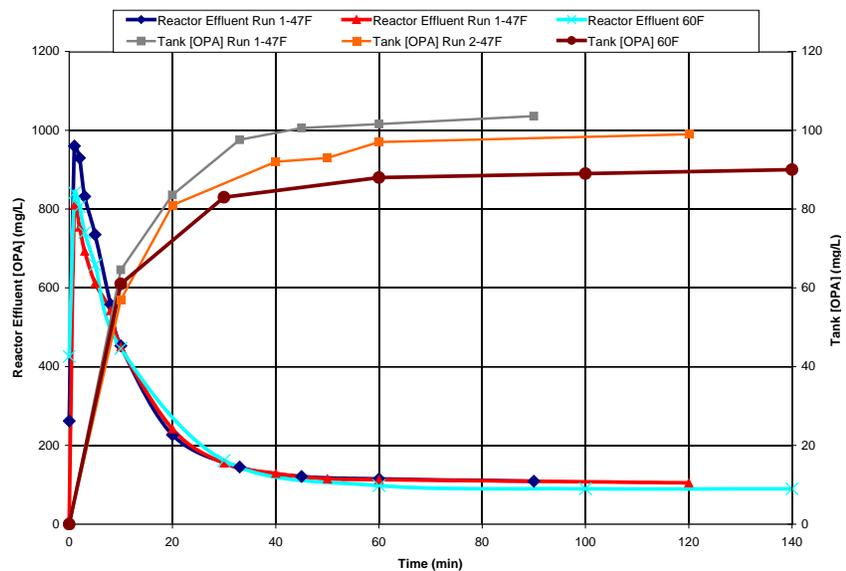


Figure 5. Results from full scale OPA delivery resin evaluation .

Assembly (NiRA) consisting of a stainless steel canister with an approximate volume of 2.0 L with flex hoses and quick disconnects to connect the canister to a payload rack location.

Development testing indicated that a 1:1 mass loading of OPA and packaging based on the flow dynamics of the canister should be used to meet the concentration requirements. The OPA delivery methodology reproducibility for a 313 liter system is shown in Figure 5.²⁸

G. OPA Rapid On-orbit Measurement System

The technique selected to accomplish on-orbit measurement of OPA concentration in the IATCS coolant was a colorimetric test strip. Since there were no commercially available products, it was determined that the development of an OPA specific test strip would be required. A search of private sector vendors led to a partnership between Hamilton Sundstrand and Branan Medical Corporation of Irvine, CA, a developer and manufacturer of a variety of test strip products. Test strip requirements included materials compatibilities (coolant chemistry, sample bag, etc.), OPA concentration range (0 to 200 mg/L), shelf life (1 year) and a reaction time not to exceed 8 - 10 minutes. Branan produced a colorimetric test strip that met all requirements and satisfied all quality and safety concerns to support maintenance of OPA in the IATCS.

The test strips consist of a proprietary chemistry utilizing a para-rosaniline indicator that changes color to indicate a corresponding OPA concentration.²⁸ Test strips are packaged into Teflon bags with a sample volume of 5 ml. After the indicator pad is submerged in the coolant sample for 7 minutes and allowed to air dry for 3 minutes, the strip is compared to a lot specific color chart within 2 minutes (Figure 6).²⁸

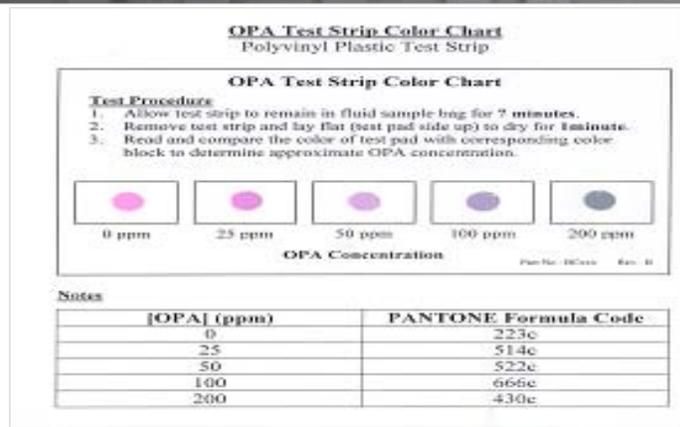
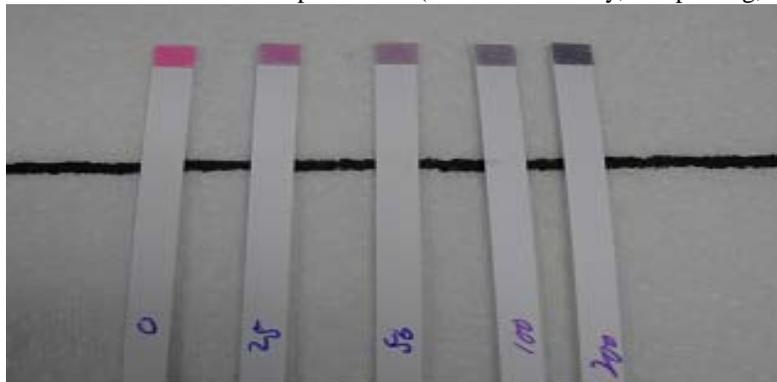


Figure 6. Results from full scale OPA delivery resin evaluation .

H. OPA Removal from the IATCS

The primary purpose of a removal resin was to be able to remove OPA from the IATCS in the event of a sustained leak to preserve crew health and safety or if adverse system effects were detected from OPA utilization in the future. Requirements included the capacity to remove 95% ± 5% of the determined OPA concentration without altering coolant alkalinity, no significant addition of leachate ions including chloride and other halogens known to increase pitting corrosion, no significant contribution of particulates, and no contribution of assimilable organic carbon.²⁸

Six candidate materials were selected for comparative evaluations including two activated carbons and four polymeric resin materials. These materials were selected for testing based upon their physical properties (i.e. pore size/distribution, surface characteristics, effective size, etc.), which play an important role in the adsorption mechanism. Each candidate was washed and sieved to its respective uniform size prior to testing. The first screening test of the candidate materials was an adsorption isotherm. The isotherm was conducted by exposing varying mass samples of each material (2 carbons and 4 resins) to an IATCS medium containing a constant OPA concentration (OPA = 210 mg/L). The samples were mixed for 24 hours after which time the fluid was analyzed for OPA, ions, pH, and surface tension (Figure 7).²⁸

A synthetic resin material labeled as R4 in Figure 6 (Ambersorb 572[®]) was chosen as the flight candidate since it had approximately twice the capacity for OPA than the activated carbons tested as shown in Figure 7. The resin material has a much more porous structure, resulting in higher surface area to facilitate organic adsorption.²⁸ The resin was also shown to remove greater than 95% of OPA degradation products from IATCS coolant but it has a higher affinity for free OPA. The resin is also a very robust material, where fines produced from attrition would be much less than in the case of an activated carbon. Ion leaching was initially a problem with this material but improved washing methodologies were implemented and successfully alleviated the issue. The evaluation of removal materials provided a flight qualified high capacity resin and a possible carbon material that would also meet system requirements.²⁸

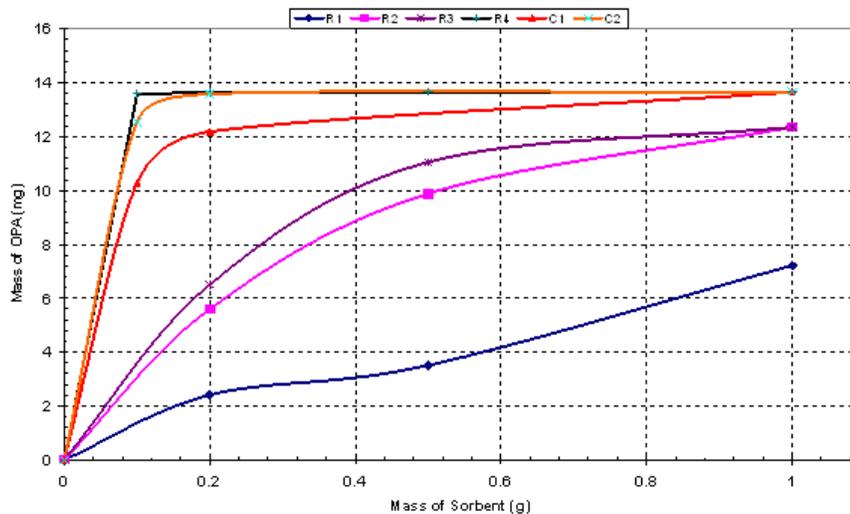


Figure 7. Results from full scale OPA delivery resin evaluation .

The removal resin had to be packed wet since the wetting process generates a significant amount of gas due to the porous nature of the resin. Shelf life and stability of the packed wet beds became an issue due to the growth of microorganisms during storage. To increase shelf life, the resin was autoclaved wet at 121°C and 15 psi for 1 hour and aseptically packed in the canister which was disinfected with a 1% OPA solution in sterile DI water.²⁸

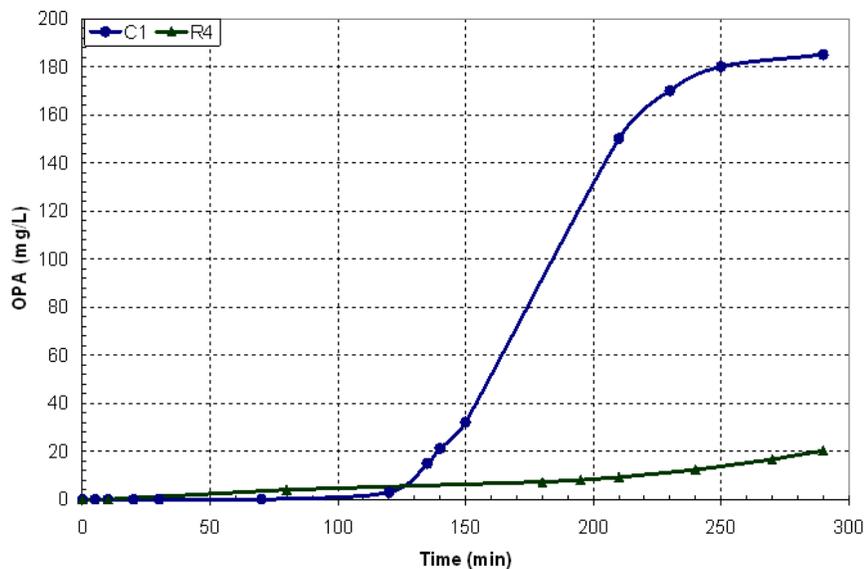


Figure 8. Scaled column breakthrough studies for selected OPA removal materials (C1 and R4)

III. On-orbit OPA Implementation and Maintenance

The OPA Delivery and Removal resins were flown on flight STS-120 aboard Discovery. Once on-board the ISS, the Delivery Resin was implemented into the US Lab IATCS on November 3, 2007. The canister was placed in a by-pass line and flow was initiated and remained constant at 400 lb/hr for 16 hours. This gave sufficient time for the OPA to be delivered to the coolant medium of the IATCS. Upon successful delivery of OPA to the coolant, the OPA test strips were used by ISS crew members to determine the actual coolant OPA concentration. Readings were taken and reported back to ground personnel as 100 mg/L. Grab samples were also taken and returned to earth on STS-120 for further analysis. The ground analysis showed the actual concentration on-orbit OPA concentration to be 149 ppm. While not detrimental to the system, the specification was exceeded and because this was substantially higher

than the targeted 100 mg/L-OPA concentration, an investigation was launched. It was determined that the volume of the US Lab IATCS was lower than that specified for the OPA Delivery Resin volume. An initial working volume of $313 \pm 10L$ was specified as a requirement and the OPA delivery resin used during STS-120 was packaged for a 313 L loop volume. Instead the loop volume was closer to 272 L on-orbit during the biocide implementation since flow was not established to all payloads. This factor, along with a possible higher elution efficiency of OPA from the resin material due to zero-g effects was determined to be responsible for the higher final OPA concentration.

The addition of 100 ppm OPA to the US Lab immediately reduced the level of planktonic heterotrophic bacteria from approximately $1E+06$ CFU/100 ml to <20 CFU/100 ml. No viable microorganisms were recovered in samples collected through April, 2008. This is due to OPA's inhibition of visible growth of bacteria as long as an inhibitory level of 10 ppm OPA was present in the coolant.

The OPA concentration in a grab sample collected from the US Lab on June 1, 2008 and returned on Flight 1J was determined to be less than the detection limit (<1 ppm OPA). Re-growth of microorganisms had occurred, the measured 7 day heterotrophic plate count on R2A incubated at $28^{\circ}C$ for 7 days was $2.2E+07$ CFU/100 ml. Species consisted of *Pseudomonas mendocina*, *Sphingobium yanoikuyae*, and an unidentified gram negative rod.

The initial degradation rate of OPA in the US Lab was approximately 1 ppm per day. This was significantly higher than degradation rate of OPA in the Systems Test (0.26 ppm/day). The higher on-orbit OPA degradation rate may be explained by the greater amount of material surface area in the US Lab as compared to the Systems Test with active sites catalyzing oxidation at $pH > 9.0$.

The concentration of OPA in Node 2 in the moderate temperature loop and low temperature loop was determined to be approximately 77 ppm at the time of system closeout for flight in February 2007. OPA concentration in Node 2 grab samples returned from on-orbit after system startup was 31 ppm on 2/8/2008, 23 ppm on 3/11/2008, and 6 ppm on 6/9/2008. The degradation rate was identical to the Systems Test at <0.3 ppm/day. This may be due to a high level disinfection of Node 2 that was performed prior to OPA addition with 250 ppm glutaraldehyde that served to reduced active sites for OPA reaction on materials and biofilms. And no microbiological re-growth has occurred in Node 2 with concentrations of OPA at < 1 ppm.

Figure 9 graphs the OPA concentrations for each on-orbit ISS element at closeout before launch. The concentration of OPA in Columbus Module and the Japanese Experiment Module (JEM) before closeout for flight

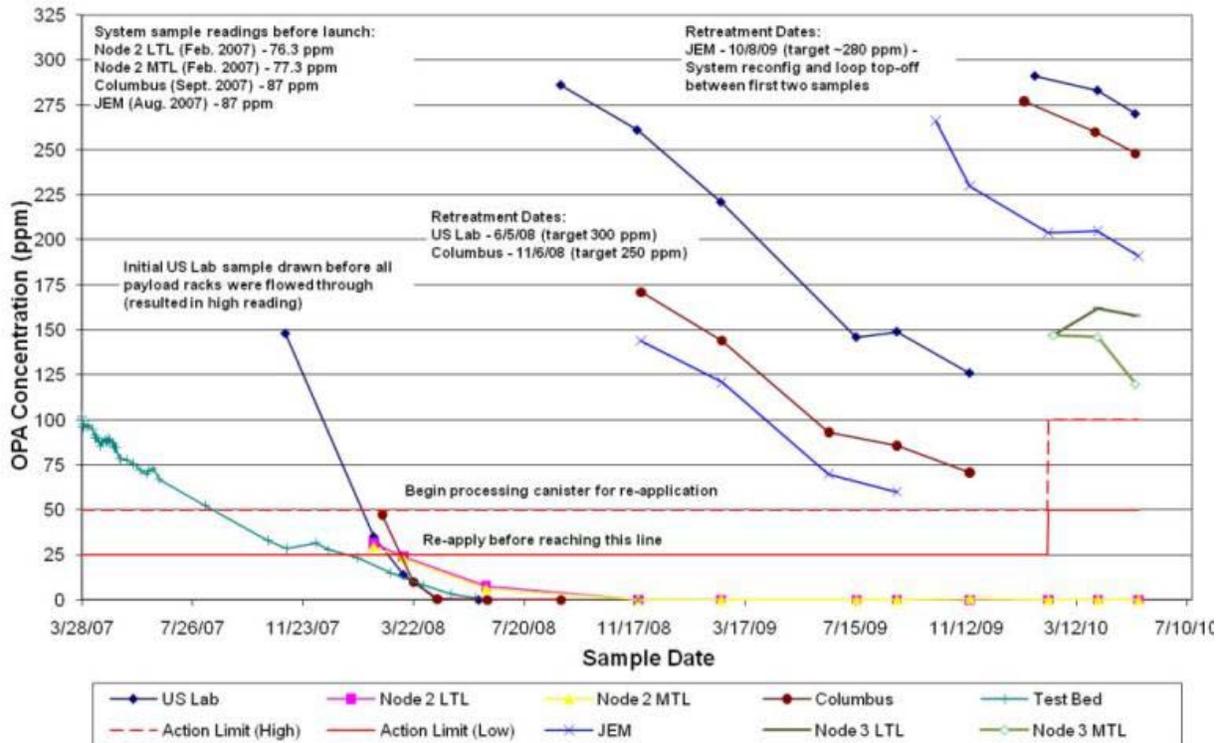


Figure 9. On-orbit addition and maintenance of OPA in ISS elements

was determined to be approximately 87 ppm. OPA degradation rates of greater than 1 ppm/day occurred in both modules after on-orbit operations were initiated in 2008. Node 3 deployed in February 2010 had approximately 150 ppm OPA measured at system startup.

Due to the rapid on-orbit reduction of OPA in the US Lab, Columbus Module, and JEM, a decision was made to increase the concentration in the US Lab, Columbus, and Node 3 to 300 ppm since logistics could not support addition of 100 ppm every 3 months. The concentration of OPA in the JEM was only increased to 200 ppm pending material testing at 300 ppm OPA. Figure 9 also show retreatment dates for the US Lab (June 5, 2008), Columbus (November 6, 2008), and the JEM (October 8, 2009).

The addition of 200 – 300 ppm OPA has resulted in an average degradation rate of approximately 0.3 ppm/day in all elements. The reduction in OPA degradation rates may be due to passivation of nickel alloys and feedback equilibration due to gradual development of higher concentrations of 2-formyl benzoic acid as compared to OPA. Based on these OPA degradation rates, canisters are processed for re-application at 100 ppm and re-application is performed before OPA decreases to < 50 ppm.

OPA removal via the Antimicrobial Removal Assembly (683-63436-3) was developed as a contingency capability and has not been performed in the on-orbit system at the time of this paper release.

IV. Conclusion

OPA has effectively inhibited the growth and recovery of viable microorganisms in the IATCS coolant when the concentration of OPA is greater than the minimum inhibitory concentration of 10 ppm. Re-growth of bacteria in the US Lab occurred when the concentration of OPA dropped below the inhibitory concentration. Standard practice for biocide 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.¹⁵ However precautions regarding system material compatibility, toxicity, and impacts on ECLSS prevented an initial application higher than 100 ppm OPA.

Hardware is not available to re-apply OPA to every on-orbit loop at a rate of once every 3 months that was seen with the initial addition of the OPA to the US Lab. These logistical constraints required an increase to the as-circulated OPA concentration from 25 – 105 ppm to 25 – 325 ppm. The increase in the as-circulated concentration of OPA to 200 – 300 ppm reduced the degradation rate in each coolant loop to approximately 0.3 ppm/day and also reduced the reapplication requirements to once every 1 – 2 years in each element.

The development, testing, flight qualification, and implementation of OPA in the IATCS has culminated in a safe, compatible, effective, and sustainable method to control microbiological growth in the ISS IATCS to prevent microbial impacts to coolant flow, heat transfer, corrosion, and crew health and safety. Maintainability of the OPA in the IATCS was made possible by the development of a safe and effective method to add OPA using a resin to limit exposure of the crew to concentrated OPA, development of a rapid, colorimetric test strip to measure OPA from 0 – 200 ppm, and development of a removal resin to effectively remove OPA and OPA degradation products in the event of a leak. This systematic approach for biocide qualification and implementation may be adapted for future space applications.

Acknowledgments

The work described in this paper was performed by NASA, Boeing, and subcontractors under the auspices of the International Space Station contract, NAS15-10000. The authors wish to express their sincere thanks to Johnny Golden, Ph.D. of Boeing and Bill Oehler of Hamilton Sundstrand for their support on material compatibility testing and Natalee Weir of Boeing for microbiological testing. The authors also express gratitude to Mike Holt of NASA MSFC and Brad Glasscock of Boeing for their project support and co-leadership of the International Space Station IATCS System Problem Resolution Team.

References

¹Stegman, T. W. and Wilson, M. E., “ISS Internal Active Thermal Control System (IATCS) Aqueous Carbon Dioxide Chemistry,” ISS IATCS System Problem Resolution Working Group Meeting, 2008.

²Stegman, T. W. and Wilson, M. E., “ISS Internal Active Thermal Control System (IATCS) Alkalinity in an Open Carbon Dioxide System,” ISS IATCS System Problem Resolution Working Group Meeting, 2008.

³Stegman, T. W., Cole H. E., Srinivasan, S. and Wilson, M. E., “ISS Internal Active Thermal Control System (IATCS) Sodium Bicarbonate/Carbonate Buffer in an Open Aqueous Carbon Dioxide System,” ISS IATCS System Problem Resolution Working Group Meeting, 2008.

⁴Block, S. S. (ed.), *Disinfection, Sterilization, and Preservation*, 5th ed., Lippincott Williams & Wilkins, Philadelphia, PA, 2001.

⁵Pohlman, M. J., Varsik, J., Steele, J., Golden, J. L., Boyce, W. E., and Pedley, M. D., “Corrosion Testing of Brazed Space Station IATCS Materials,” *SAE Technical Paper* 2004-01-2471, 2004.

- ⁶Characklis, W. G., Trulear, M. G., and Stathopoulos, N., "Fundamental Considerations in Biofouling Control," *Cooling Technology Institute Technical Paper* TP80-02, 1980.
- ⁷Willis, C. E. and Schultz, J. R., "Spacecraft Water System Disinfection Technology: Past, Present, and Future Needs," 17th Intersociety Conf. Environ. Sys., *SAE Technical Paper* 871487, 1987.
- ⁸Lappin-Scott, H.L. and Costerton, W. J. "Bacterial Biofilms and Surface Fouling", *Biofouling*, Vol. 1, 1989, pp. 323-342.
- ⁹Mittelman, M. W., Mitchell, R., and Ridgway, H., "Assessment of ISS IATCS Loop Microbiological Control Options," NASA RFP PS410-06/09/04, 2004.
- ¹⁰Wilson, M., Cole, H., Weir, N., Roman, M., Steele, J., and Lukens, C., "Selection of an Alternate Biocide for the International Space Station Internal Active Thermal Control System Coolant Loops," *SAE Technical Paper* 2003-01-2568, 2003.
- ¹¹Wilson, M., Cole, H., Weir, N., Oehler, B., Steele, J., Varsik, J., and Lukens, C., "Selection of an Alternate Biocide for the ISS Internal Thermal Control System Coolant – Phase II," *SAE Technical Paper* 2004-01-2472, 2004.
- ¹²Pyle, B. H., Broadway, S. C., Pulcini, E., Ford, T., Camper, A. K., and Sturman, P., "Assessment of ISS IATCS Loop Microbial Control Options," Montana State University – Bozeman, 2004.
- ¹³Weir, N. E., Hutton, A. L., Tucker, C. D., Wilson, M. E. and Cole, H. E., "Comparison of the Effects of Different Nutrients on Aldehyde Antimicrobials," The Boeing Company 2005-09-07-01529, 2005.
- ¹⁴Nickerson, C. A., Ott, C. M., Wilson, J. W., Ramamurthy, R., and Pierson, D. L., "Microbial Responses to Microgravity and Other Low-Shear Environments," *Microbiol. and Molecular Biol. Rev.*, Vol. 68, No. 2, 2004, pp. 345-361.
- ¹⁵Black, J. G. (ed.), *Microbiology: Principles and Explorations*, 6th ed., 2004, Wiley.
- ¹⁶Zhu, P. C. (ed.), *New Biocides Development, The Combined Approach of Chemistry and Microbiology*, ACS Symposium Series 967, 2007, American Chemical Society, Washington D.C.
- ¹⁷McDonnell, G. and Russell, A. D. *Antispetics and Disinfectants: Activity, Action, and Resistance*, Vol. 12, No. 1, 1999, pp. 147-179.
- ¹⁸Oehler, B., "Non-Metallic Samples in Candidate Biocide Coolant Mixture Report", HOU-011 Rev. Basic, Hamilton Sundstrand, 7/21/06.
- ¹⁹Varsik, J., "Material Compatibility Testing Using Ortho-phthaldehyde (OPA) at Introduction and Use Concentrations", HOU-009 Rev. Basic, Hamilton Sundstrand, 7/06/06.
- ²⁰Varsik, J., "Final Report for Metallic Material Compatibility Testing Using Orthophthalaldehyde (OPA) Up To and Including 600ppm", HOU-1625 Rev Basic, Hamilton Sundstrand, 3/10/09.
- ²¹Hamilton Oehler, B., "Final Report for Material Compatibility Testing Using Orthophthalaldehyde (OPA) at 600ppm", HOU-1624 Rev Basic, Hamilton Sundstrand, 03/10/09.
- ²²Coleman, M. E., "Toxicological Assessment of IATCS Coolant Biocides," Memorandum 632, March 4, 2005.
- ²³Coleman, M. E., "Toxicological Rating of Ortho Phthaldehyde (OPA) Biocidal Agent to be Used in the International Space Station (ISS) Internal Active Thermal Control," Memorandum TOX-MC-2007-TBD, August 27, 2007.
- ²⁴Coleman, M. E., "Toxicological Rating of New Levels of Ortho Phthaldehyde (OPA) Biocidal Agent to be used in the Antimicrobial Applicator (AmiA), connected to the International Space Station (ISS) Internal Active Thermal Control System (IATCS) Coolant Loop," Memorandum TOX-MC-2008-11, June 27, 2008.
- ²⁵Garcia, H. D., "Toxicological Assessment of Solid Ortho Phthalaldehyde (OPA) from ITCS Fluid," Memorandum TOX-HG-2009-09, April 30, 2009.
- ²⁶Perry, J. L., "Compatibility of the Candidate ortho-Phthalaldehyde Internal Thermal Control System Antimicrobial Additive with the International Space Station's Environmental Control and Life Support System," NASA Engineering Analysis Report, September 14, 2005.
- ²⁷Macatangay, A. V., "Re-evaluation of the Compatibility of ortho-Phthalaldehyde ITCS Antimicrobial Additive with ISS ECLS System," March 10, 2008.
- ²⁸Rector, T., Steele, J., and Wilson, M., "ISS IATCS Coolant Loop Biocide Implementation," SAE Technical Paper 2008-01-2159, 2008.